



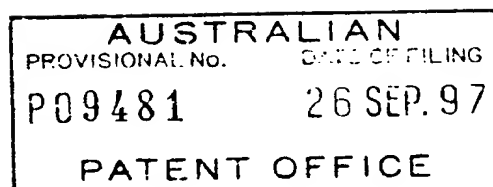
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I, LEANNE MYNOTT, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PO 9481 for a patent by THE NATIONAL UNIVERSITY OF SINGAPORE filed on 26 September 1997.

WITNESS my hand this
Twenty-ninth day of November 1999

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The National University of Singapore

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Diagnosis of Parasites"

The invention is described in the following statement:

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DIAGNOSIS OF PARASITES

The present invention relates generally to novel genetic sequences isolated from a parasitic protozoa which infects humans and other animals and the uses of said sequences as
5 diagnostic agents for the detection of said protozoa in a biological sample. In particular, the present invention provides genetic sequences of the extrachromosomal genetic elements of the malaria agents *Plasmodium berghei*, *Plasmodium vivax* and *Plasmodium malariae* and synthetic oligonucleotide derivatives, homologues, analogues and fragments thereof. The genetic sequences of the present invention are particularly useful in the diagnosis,
10 prophylactic treatment and therapeutic treatment of humans and other animals which are capable of being infected by or are actually infected by protozoa such as *Plasmodium ssp.*, for example *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. cynomolgi*, *P. gonderi*, *P. (Hepatoctysis) kochi*, *P. inui*, *P. knowlesi*, *P. reichenowi*, *P. rodhaini*, *P. schwetzi*, *P. cathemerium*, *P. elongatum*, *P. relictum*, *P. lophurae*, *P. gallinaceum*, *P. chabaudi*, *P. yoelii*,
15 or *P. berghei*, amongst others. The invention provides further, a novel, reliable diagnostic assay for the detection of *Plasmodium ssp.* in humans and animals.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence identity numbers (SEQ ID Nos.) for the
20 nucleotide and amino acid sequences referred to in the specification are defined after the bibliography.

Throughout the specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply
25 the inclusion of a stated element or integer or group of elements or integers, but not the exclusion of any other element or integer or group of elements or integers.

More than fifty different species of *Plasmodium* can cause malaria in humans, monkeys, birds, fish, cattle and rodents. The development of diagnostic assays for the
30 detection of *Plasmodium* in humans and animals is therefore highly desirable.

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Human malaria, which is caused by *Plasmodium* spp., in particular *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*, remains one of the major health problems around the world.

Plasmodium vivax induces a moderate form of malaria, vivax malaria, characterized by periodic chills and fever, an enlarged spleen, anaemia, severe abdominal pain and headaches, and extreme lethargy. If left untreated, the disease tends to be self-limiting within a period of 10 to 30 days, but will recur periodically. Although the fatality rate of vivax malaria is low, the disease is highly debilitating and makes the patient more vulnerable to other diseases.

10

The incubation period ranges from 10 days to 4 weeks. Generally, paroxysms of chills and fever appear on the 14th day after the bite of an infected female anopheles mosquito. During this time the parasite has been multiplying in the liver cells of the patient. Paroxysms continue to recur every other day, as the parasite completes its 48-hour cycle of development, now in the blood. During the paroxysm, the patient first goes through a "cold stage" during which he has chilly sensations, his skin is blue, his teeth chatter and there is violent shaking. After an hour, the "hot stage" is ushered in, with a rise in temperature to as high as 107°F (41.7°C); the skin is hot and dry and the patient complains of severe headache. The fever lasts about 2 hours, and is followed by the "sweating stage", during which there is profuse perspiration, the temperature falls to normal, the headache disappears, and although weak and drowsy, the patient feels well.

20

Plasmodium ovale produces a disease very similar to vivax malaria.

25

Plasmodium malariae, the causative agent of quartan malaria, has an incubation period of 18-40 days. The paroxysms occur every 72 hours, and are longer and somewhat more severe than those accompanying vivax malaria.

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Plasmodium falciparum-induced malaria (falciparum malaria) presents oedema of the brain and lungs and blockage of the kidneys, in addition to the symptoms associated with vivax malaria. Unless treated promptly, the fatality rate of falciparum malaria is high, especially in juveniles.

5

Paroxysms associated with falciparum malaria occur irregularly after a 12-day incubation period. They are severe, and accompanied by high temperatures. The so-called cerebral algid, haemorrhagic and pernicious types of malaria represent forms of falciparum malaria with different localizations of the parasite. In the cerebral type, the onset is delirium
10 and coma, and death may occur in several hours without return to consciousness. "Black-water fever" or haemorrhagic malaria is a type in which haemolysis or dissolution of the red cells occurs, and dark urine due to the presence of haemoglobin is an outstanding feature. In the algid form, there are vomiting, diarrhea, and subnormal temperature.

15 The life cycle of the parasite and its course in the human body proceeds in the following way. The saliva of the mosquito contains the *Plasmodium* at the lance-shaped sporozoite stage of its life cycle. Upon inoculation of the host by biting, the sporozoites quickly migrate to the liver where they divide and develop into multi nucleated schizonts. Within 6 to 12 days, the schizonts disrupt and release into the blood the form known as
20 *merozoites*. Each liver cell infected by one sporozoite releases into the blood stream from 10,000 to 30,000 merozoites. These later invade the host's erythrocytes where they grow and form more schizonts which, in turn, again divide, releasing more merozoites into the blood stream to repeat the cycle. The principal symptoms of malaria are associated with the rupture of the schizonts, the periodic lysis of the blood cells with release of merozoites and toxic
25 wastes which cause the regular fevers and chills of malaria.

Neither vector control measures nor immuno or chemoprophylaxis have proven effective in eradicating the disease. Thus, more than ever, chemotherapy appears to be crucial in dealing with both the prevention and treatment of malaria. However, presently used drugs
30 are constantly losing their efficacy due to the development of drug resistance by the parasite.

For example, drug resistance of *Plasmodium falciparum* to chloroquine has occurred in Bangladesh, Brazil, Burma, Colombia, Ecuador, Guyana (French), Guyana, India, Indonesia, Kampuchea, Malaysia, Nepal, Pakistan, Panama, Philippines, Surinam, Thailand, Venezuela, and Vietnam, amongst others. Therefore, the design of novel drugs is urgent.

5

Targets for drug design are generally nuclear-encoded gene products. However, inter-specific and developmental variation in nuclear gene expression has reduced the general efficacy of drugs which target such nuclear-encoded gene products.

10 Diagnosis of malaria is generally made by microscopic examination of blood films taken during episodes of fever, when the parasites may be seen. In general, the *Plasmodium* parasite is detected microscopically by examining finger prick blood samples for the presence of the morphologically distinct parasite using Giemsa stain solution (Shute *et al.*, 1980). This needs to be done by an experienced microscopist since *Plasmodium falciparum* and
15 *Plasmodium vivax* are morphologically similar, albeit not identical. In view of the distinct epidemiologies of *P. falciparum* compared to *P. vivax*, it is important that diagnosis of infection by these species have a low error rate. Any incorrect diagnosis of falciparum malaria, for example, may be fatal for the patient. The microscopic technique is limited in so far as the method is slow and specialised personnel is required to perform the technique.

20

A variation of the standard microscopic assay, the quantitative buffy coat (QBC) technique is based upon the ability of parasite nucleoproteins to absorb acridine orange and fluoresce (Wardlaw *et al.*, 1983). The fluorescent nucleoproteins are readily visible against a background of non-fluorescent red blood cells. Although the method is more sensitive than
25 the standard microscopic assay, it suffers from the disadvantages associated with the standard microscopic assay. Furthermore, the requirement of costly fluorescence microscopes and centrifuges to perform the QBC assay, renders the method unrealistic in resource-limited settings which often lack even electricity.

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Immunological tests, for example the ParaSight™ F test (Becton Dickinson) and the similar ICT Malaria P.f. test (ICT Diagnostics) detect the *Plasmodium falciparum* histidine-rich protein HRP2 in blood samples derived from patients. A major drawback associated with such methods is that they require *Plasmodium falciparum* gene expression to occur before the
5 organism can be detected. Furthermore, as considerable variation in gene expression can occur between *Plasmodium ssp.*, these tests tend to be species-specific. For example, the ParaSight™ F test (Becton Dickinson) and ICT Malaria P.f. test (ICT Diagnostics) are specific for *Plasmodium falciparum* only and incapable of detecting other species. Furthermore, these tests, in particular the ParaSight™ F test (Becton Dickinson), are subject
10 to a high proportion of false-negative detections, such that a higher than acceptable frequency of patients infected with a *Plasmodium ssp.* go undetected.

Immunological techniques such as the enzyme-linked immunosorbent assay (ELISA) or the radio immunoassay (RIA) which detect genus- and species-specific parasite antigens
15 also exist. However, such methods are constrained by immunological cross-reaction between parasite and host antigens on the one hand and between parasite antigens and antigens derived from other microorganisms on the other hand. As a consequence, the susceptibility of immunological methods to false positive detection of *Plasmodium* is high. As already mentioned above, species-specific detection methods lead to a large number of false-negative
20 detections.

Furthermore, as different *Plasmodium* antigens are expressed at different developmental stages, immunological techniques may only detect the parasite at certain stages of development. Such antigenic diversity displayed by *Plasmodium* is a major obstacle to the
25 application of immunological techniques. In addition, radioisotope-based assays such as the RIA are impractical for field use. Immunological methods cannot distinguish between past and present infections.

State-of-the art diagnostic assays, which rely on the detection of *Plasmodium* genomic
30 DNA in a sample, are species-specific and not capable of general application for any

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Plasmodium ssp., in part because there is considerable variation in genomic DNA between *Plasmodium* species, such variation precluding the simultaneous detection of several *Plasmodium ssp.* in a single biological sample or alternatively, the use of a single DNA-based assay for the detection of any *Plasmodium ssp.* in a biological sample derived from a human
5 or animal subject suspected of carrying the parasite.

As a consequence of the foregoing, there is a high demand for a reliable and simple technology for the diagnosis of *Plasmodium* in human and animal tissues.

10 *Plasmodium ssp.* possess additional genomes with potentially crucial functions (Wilson *et al.*, 1991). Until the present invention, very little was known about this extrachromosomal material. Furthermore, the function of the extrachromosomal plastid element in the protozoans remains to be determined. To date, there is no clear evidence for DNA replication or functionally active gene products from the plastid element.

15

In work leading up to the present invention, the inventors have discovered that the molecular composition, physical arrangements and nucleotide sequences of the extrachromosomal plastid-like element and mitochondrial element are highly conserved in different *Plasmodium ssp.*

20

The inventors have utilised the high degree of homology between different *Plasmodium ssp.* in the design of reliable, genera-specific or species-specific diagnostic assays for the detection of *Plasmodium*. The diagnostic assays described herein provide a significant advantage over currently employed assays based upon the detection of *Plasmodium* genomic
25 DNA.

Furthermore, the inventors have discovered that the assays described herein provide the added advantage of excluding the high frequency of false negative detection of *Plasmodium* in a biological sample to a greater degree than known diagnostics.

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The inventors further contemplate the use of polypeptides encoded by the extrachromosomal plastid-like element, and their homologues, analogues and derivatives, as targets for drug design and in the development of anti-malarial vaccines.

5 Accordingly, one aspect of the invention provides a diagnostic assay for the detection of *Plasmodium* in a biological sample derived from a human or animal subject, said assay comprising the detection of a *Plasmodium* extrachromosomal genetic element or a homologue, analogue or derivative thereof in said sample.

10 In an alternative embodiment, the invention provides a diagnostic assay for the detection of *Plasmodium* in a biological sample derived from a human or animal subject, said assay comprising the steps of hybridising a *Plasmodium* *ssp.* extrachromosomal genetic element probe or a homologue, analogue or derivative thereof to said sample and then detecting said hybridisation using a detection means.

15 According to this aspect, the *Plasmodium* detected using the invention may be any species of *Plasmodium* which carries an extrachromosomal genetic element.

20 In a preferred embodiment, the *Plasmodium* being detected is selected from the list comprising *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. cynomolgi*, *P. gonderi*, *P. (Hepatocytis) kochi*, *P. inui*, *P. knowlesi*, *P. reichenowi*, *P. rodhaini*, *P. schwetzi*, *P. cathemerium*, *P. elongatum*, *P. relictum*, *P. lophurae*, *P. gallinaceum*, *P. yoelii*, or *P. berghei*, amongst others.

25 In a more particularly preferred embodiment however, the present invention is useful for the detection of a *Plasmodium* in biological samples derived from humans and the *Plasmodium* in such cases is selected from the list comprising *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, amongst others.

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The term "biological sample" as used herein shall be taken to refer to any organ, tissue, cell, exudate, nucleic acid, protein, nucleoprotein or other material which is derived from a living or once-living organism. Accordingly, biological samples may be mosquito or other vectors of *Plasmodium* ssp., human or animal tissue, blood or derivatives of blood and
5 blood products, amongst others. A biological sample may be prepared in a suitable solution, for example an extraction buffer or suspension buffer. The present invention extends to the diagnosis of biological solutions thus prepared, the only requirement being that said solution at least comprises a biological sample as described herein.

10 The biological sample to be tested according to the invention, is derived from a human or animal species, in particular a human or animal which is capable of being infected by a *Plasmodium*. A particular advantage of the present invention is that it may be readily adapted to facilitate the analysis of any biological sample derived from a human or other animal. Those skilled in the relevant art will know how to modify the assay of the invention for the
15 purposes of adapting said assay to the analysis of different biological tissues, where relevant or indicated, without any undue experimentation.

In a particularly preferred embodiment, the biological sample may be derived from the blood tissue of a human or animal subject, or cells, nucleic acid molecules and exudates
20 derived therefrom, for example buffy coat, plasma, DNA or RNA, amongst others. The use of dried blood spots derived from human subjects as biological samples for the performance of the assays described herein is particularly contemplated by the invention.

The term "extrachromosomal genetic element" shall be taken to refer to any nucleic
25 acid molecule, in particular DNA or RNA, which comprises a part of the complete genetic material of a *Plasmodium* ssp. but which does not comprise a part of a *Plasmodium* ssp. chromosome or a direct gene product thereof. An extrachromosomal genetic element of a *Plasmodium* ssp. may or may not replicate independently of the *Plasmodium* genome, such that the copy number of said genetic element may vary between *Plasmodium* cells.

30

Accordingly, a *Plasmodium* extrachromosomal genetic element as hereinbefore defined may be a linear or circular DNA molecule. In this regard, a linear DNA molecule may resemble, at the nucleotide sequence level at least, mitochondrial DNA (Suplick *et al*, 1988), while the circular DNA molecule in a *Plasmodium* resembles a vestigial plastid genome
5 (Gardner *et al*, 1991; Howe *et al*, 1992).

The present inventors have shown herein that the malaria parasites harbour two extrachromosomal DNAs. One of these is a small 6 kb molecule which encodes three classical mitochondrial protein coding genes, attesting to its identity. The other is a circular
10 molecule resembling the remnant of an algal plastid genome. The mitochondria DNA of *Plasmodium* species is very short; about 6 kb and codes for three proteins, namely cytochrome *c* oxidase subunits I (cox I) and III (cox III), and cytochrome *b* (cyt *b*) as well as fragments of ribosomal RNA genes.

15 In a preferred embodiment of the invention, the *Plasmodium* extrachromosomal genetic element is a plastid DNA molecule comprising approximately 30-35kb of nucleotides in length.

In an alternative preferred embodiment, the *Plasmodium* extrachromosomal genetic
20 element is mitochondrial DNA or mitochondrion-like DNA comprising approximately 6 kb in length.

Furthermore, the diagnostic assay of the present invention is useful for the detection of a *Plasmodium* extrachromosomal genetic element or a *Plasmodium*-derived
25 extrachromosomal genetic element, regardless of whether or not said genetic element expresses or is capable of expressing a polypeptide product.

The term "*Plasmodium*-derived" as used herein shall be taken to refer to an integer which, although it originates from a *Plasmodium ssp.* is not necessarily present in its natural
30 state. For example, an extrachromosomal genetic element may be derived from a *Plasmodium*

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ssp. if it has been purified or partially purified and/or modified by digestion with restriction endonucleases or other DNA-modifying enzymes, to produce an analogue or derivative molecule.

5 The *Plasmodium* extrachromosomal genetic element probe may be a mitochondrion or mitochondrion-like molecule or alternatively a plastid or plastid-like molecule, derived from a *Plasmodium ssp.* which is capable of infecting a human or animal subject.

It is preferred that the extrachromosomal genetic element be derived from a species
10 of *Plasmodium* other than *P. falciparum*.

In a particularly preferred embodiment, the extrachromosomal genetic element probe or a homologue, analogue or derivative thereof, is derived from *Plasmodium berghei*, *Plasmodium vivax*, or *Plasmodium malariae*, *Plasmodium chabaudi*, or *Plasmodium yoelii*,
15 amongst others.

However, in a more particularly preferred embodiment of the invention, the extrachromosomal genetic element probe is at least 95% identical to one or more of the sequences set forth in SEQ ID Nos: 1-15 or alternatively, encode a sequence of amino acids
20 set forth in Figure 10, or a complementary nucleotide sequence, or a homologue, analogue or derivative thereof.

Alternatively, the extrachromosomal genetic element probe is capable of hybridising under high stringency conditions to one or more of the sequences set forth in SEQ ID NOS:
25 1-15 or to the *Plasmodium vivax* or *Plasmodium malariae* sequences set forth in Figure 9 or to a primer sequence set forth in Figure 10, or a complementary nucleotide sequence or a homologue, analogue or derivative thereof.

In a further alternative embodiment, the *Plasmodium ssp.* extrachromosomal genetic
30 element probe preferably comprises a sequence of nucleotides of at least 15 nucleotides, more

preferably at least 25 nucleotides, even more preferably at least 50 nucleotides and even more preferably at least 100 nucleotides or 500 nucleotides derived from the sequence set forth in SEQ ID NOs:1-4 or to the *Plasmodium vivax* or *Plasmodium malariae* sequences set forth in Figures 9 or 10, or a complement or a homologue, analogue or derivative thereof.

5

In a most particularly preferred embodiment, the extrachromosomal genetic element probe comprises a nucleotide sequence set forth in any one or more of SEQ ID NOS: 1-15 or the *Plasmodium vivax* or *Plasmodium malariae* sequences set forth in Figures 9 or 10, or a complementary nucleotide sequence, or a homologue, analogue or derivative thereof.

10

For the purposes of nomenclature, the nucleotide sequences set forth in SEQ ID NOs:1-4 correspond to one strand of the PSI-PL470, PLH-PPH, PRB and PWQ genes, respectively, of the 30.7 kb *Plasmodium berghei* plastid. The inventors have shown that the extrachromosomal genetic element is transcriptionally-active, using reverse transcription
15 polymerase chain reaction (RT-PCR), and encodes organelle-like rRNAs, tRNAs, ribosomal proteins and RNA polymerase subunits, amongst others.

The nucleotide sequences set forth in SEQ ID Nos: 5-10 correspond to synthetic oligonucleotide sequences derived from the *Plasmodium* extrachromosomal genetic element
20 of *P. berghei*.

The nucleotide sequences set forth in SEQ ID Nos: 11-16 correspond to synthetic oligonucleotide sequences derived from the *Plasmodium vivax* mitochondrial coxI gene, while the nucleotide sequences set forth in SEQ ID Nos: 13-15 correspond to synthetic
25 oligonucleotide sequences derived from the *P. falciparum* mitochondrial coxI gene.

The nucleotide sequence Pm1/S and Pm38/S in Figure 9 relate to the extrachromosomal genetic element in two *P. malariae* isolates and Po35/S and Po36/S relate to the extrachromosomal genetic element of two *P. ovale* isolates.

30

The nucleotide sequences designated Pv12/P, Pb13/P, Pv15/I, Pv16/L, Pv17/S and Pv86/C in Figure 9 relate to extrachromosomal genetic element sequences of different *P. vivax* isolates.

5 For the present purpose, "homologues" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

10

"Analogues" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic
15 acid molecule, for example carbohydrates, radiochemicals including radio nucleotides, reporter molecules such as, but not limited to biotin, DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any
20 isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or
25 multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are characterised by the removal of one or more nucleotides from the nucleotide
30 sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the

sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

The present invention encompasses all such homologues, analogues or derivatives of
5 a *Plasmodium* extrachromosomal genetic element, subject to the proviso that said homologues, analogues or derivatives are useful in the performance of at least one assay format as described herein.

The inventors have discovered that the extrachromosomal genetic element of
10 *Plasmodium* is particularly useful as a marker of *Plasmodium* infection in a human or animal subject, because the detection of said element is not subject to the disadvantages of other assay methods, in particular the prevalence of false negative detection. As a consequence, fewer numbers of *Plasmodium*-infected hosts escape detection, by screening such hosts for the presence of the extrachromosomal genetic element according to the embodiments
15 described herein (1% or less false negative detection compared to 3% or more for other methods), than by screening for the presence of other *Plasmodium*-expressed genes or by screening for the expression products of said genes.

Furthermore, the present invention is a procedure for assaying or identifying
20 *Plasmodium* in a biological sample, in particular a biological sample which comprises a dried blood spot.

The present invention clearly contemplates diagnostic assays which are capable of both genera-specific or species-specific detection. Accordingly, in one embodiment, the
25 *Plasmodium ssp.* extrachromosomal genetic element probe or homologue, analogue or derivative thereof comprises DNA capable of being used to detect multiple *Plasmodium ssp.* In an alternative embodiment, the *Plasmodium ssp.* extrachromosomal genetic element probe or homologue, analogue or derivative thereof comprises DNA capable of being used to detect a particular *Plasmodium ssp.*

The inventors have discovered further that the coding region of a *Plasmodium* extrachromosomal genetic element is highly-conserved in different *Plasmodium ssp.*, while there is much more variation at the nucleotide level in the non-coding regions. Whilst not being bound by any theory or mode of action, the more highly conserved sequences in the
5 extrachromosomal genetic element derived from a particular species of *Plasmodium* are particularly useful as genera-specific probes for the detection of any *Plasmodium*, while the less-conserved sequences of said element may be useful as species-specific probes for the detection of a sub-group of *Plasmodium*, for example a sub-group which infects humans or primates as opposed to other animals, or which induces a specific form of malaria in humans.

10

The present inventors have also shown herein that certain sequences of the *Plasmodium* cytochrome *c* oxidase differ between species. Accordingly, a preferred embodiment of the present invention extends to the use of nucleotide sequences derived from the mitochondrial extrachromosomal genetic element of *Plasmodium*, more preferably derived
15 from *P. falciparum* or *P. vivax* in the diagnosis of species-specific infections by one or more of *P. malariae*, *P. ovale*, *P. vivax* or *P. falciparum*, amongst others.

Furthermore, one or more of the diagnostic assays described herein may also be adapted to a genera-specific or species-specific assay by varying the stringency of the
20 hybridisation step. Accordingly, a low or lower stringency hybridisation may be used to detect several different species of *Plasmodium* in one or more biological samples being assayed, while a high or higher stringency of hybridisation is used to detect the presence of a specific species of *Plasmodium*.

25 For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or wash carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or wash carried out in 0.1xSSC
30 buffer, 0.1% (w/v) SDS at a temperature of at least 65°C. Those skilled in the art will be

aware of equivalent reaction conditions to those described herein for defining the hybridisation stringency.

Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or wash may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification of the parameters affecting hybridisation between nucleic acid molecules, reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

The detection means according to this aspect of the invention may be any nucleic acid-based detection means, for example nucleic acid hybridisation techniques or an amplification reaction such as a polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) system. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR) and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

Wherein the detection means is a nucleic acid hybridisation technique, the *Plasmodium* extrachromosomal genetic element probe may be labelled with a reporter molecule capable of producing an identifiable signal (e.g. a radioisotope such as ^{32}P or ^{35}S or a biotinylated molecule). According to this embodiment, those skilled in the art will be aware that the detection of said reporter molecule provides for identification of the *Plasmodium* extrachromosomal genetic element probe and that, following the hybridisation reaction, the detection of the corresponding *Plasmodium* *ssp.* extrachromosomal genetic element in the biological sample is facilitated.

Wherein the detection means is an RFLP, nucleic acid derived from the biological sample, in particular DNA, is digested with one or more restriction endonuclease enzymes and the digested DNA is subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to the *Plasmodium* extrachromosomal genetic element probe as hereinbefore defined, optionally labelled with a reporter molecule. According to this embodiment, a specific pattern of DNA fragments is hybridised to the *Plasmodium* extrachromosomal genetic element probe, said pattern optionally specific for a particular *Plasmodium* spp., to enable the user to distinguish between different species of the parasite.

10

Wherein the detection means is an amplification reaction for example a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant of same, one or more nucleic acid primer molecules of at least 15 contiguous nucleotides in length derivable from the *Plasmodium* extrachromosomal genetic element probe as hereinbefore defined, or its complementary nucleotide sequence or a homologue, analogue or derivative thereof, is hybridised to the biological sample comprising nucleic acid or alternatively, to nucleic acid derived from said sample and nucleic acid copies of the *Plasmodium* extrachromosomal genetic element present in said sample or a part or fragment thereof are enzymically-amplified.

20

Those skilled in the art will be aware that there must be a sufficiently high percentage nucleotide sequence identity between the *Plasmodium* extrachromosomal genetic element probe(s) and the sequences in the template molecule to which it(they) hybridise. As stated previously, the hybridisation conditions may be varied to promote hybridisation.

25

Preferably, the *Plasmodium* extrachromosomal genetic element probe is at least 95% identical to the complement of the nucleotide sequence in the template molecule to which it hybridises. More preferably, the *Plasmodium* extrachromosomal genetic element probe is substantially the same as the complement of the nucleotide sequence in the template molecule to which it hybridises.

30

Preferably, the *Plasmodium* extrachromosomal genetic element probe is contained in an aqueous mixture of other nucleic acid primer molecules. More preferably, the nucleic acid primer molecule is in a substantially pure form.

5 According to this embodiment of the invention, the *Plasmodium* extrachromosomal genetic element probe(s) may comprise inosine, adenine, guanine, thymidine, cytidine or uracil residues or functional analogues or derivatives thereof which are capable of being incorporated into a polynucleotide molecule, provided that the resulting probe is capable of hybridising under at least low stringency conditions to a *Plasmodium* extrachromosomal
10 genetic element.

In a particularly preferred embodiment, the *Plasmodium* extrachromosomal genetic element probe comprises the sequence of nucleotides set forth in any one or more of SEQ ID Nos: 5-15 or Figures 9 or 10 or a complementary strand or a homologue, analogue or
15 derivative thereof.

In a more particularly preferred embodiment, the *Plasmodium* extrachromosomal genetic element probes are hybridised to a *Plasmodium* extrachromosomal genetic element contained in the biological sample being analysed, as probe pairs, in the combinations
20 comprising SEQ ID Nos: 5-6 or SEQ ID Nos: 7-8 or SEQ ID Nos: 9-10 or SEQ ID Nos. 11-12 or SEQ ID Nos: 11 and 13 or SEQ ID Nos: 11 and 14 or SEQ ID Nos: 11 and 15 or complementary strands, homologues, analogues or derivatives thereof.

In a most particularly preferred embodiment, the *Plasmodium* extrachromosomal
25 genetic element probes are hybridised to a *Plasmodium* extrachromosomal genetic element contained in the biological sample being analysed, as the L/L probe pair comprising SEQ ID Nos: 5-6 or SEQ ID Nos. 11-12 or SEQ ID Nos: 11 and 13 or SEQ ID Nos: 11 and 14 or SEQ ID Nos: 11 and 15 complementary strands, homologues, analogues or derivatives thereof.

The present invention particularly contemplates the use of primers as set forth in one or more of SEQ ID Nos: 11-15 as being useful in the differentiation of *Plasmodium* species as well as for detecting *Plasmodium* in a biological sample.

5 The *Plasmodium* extrachromosomal genetic element present in the biological sample, or a part or fragment thereof which is enzymically-amplified, is defined herein as a "template molecule". The template molecule may be a genetic sequence which is at least 40% identical at the nucleotide sequence level to SEQ ID Nos: 1-4 or to its complementary nucleotide sequence or the *Plasmodium vivax* or *Plasmodium malariae* sequences set forth in Figure 9
10 or Figure 10, the only requirement being that it comprises a *Plasmodium* extrachromosomal genetic element as hereinbefore defined.

Those skilled in the art will also be aware that, in one format, the polymerase chain reaction provides for the hybridisation of non-complementary *Plasmodium* extrachromosomal
15 genetic element probes to different strands of the template molecule, such that the hybridised probes are positioned to facilitate the 5'- 3' synthesis of nucleic acid in the intervening region, under the control of a thermostable DNA polymerase enzyme. As a consequence, the polymerase chain reaction provides an advantage over other detection means in so far as the nucleotide sequence in the region between the hybridised *Plasmodium* extrachromosomal
20 genetic element probes may be unknown and unrelated to any known nucleotide sequence.

In a particularly preferred embodiment, the nucleic acid template molecule comprises, in addition to other nucleotide sequences, a sequence of nucleotides derived from or contained within any one or more of the sequences set forth in SEQ ID Nos: 1-16 or a
25 complementary sequence or a homologue, analogue or derivative thereof.

In an alternative embodiment, wherein the detection means is AFLP, the *Plasmodium* extrachromosomal genetic element probes are selected such that, when nucleic acid derived from the biological sample, in particular DNA, is amplified, different length amplification
30 products are produced from different *Plasmodium ssp*. The amplification products may be

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subjected to electrophoresis, transferred to a solid support such as, for example, a nylon or nitrocellulose membrane, and hybridised to the *Plasmodium* extrachromosomal genetic element probe as hereinbefore defined, optionally labelled with a reporter molecule. According to this embodiment, a specific pattern of amplified DNA fragments is hybridised
5 to the *Plasmodium* extrachromosomal genetic element probe, said pattern optionally specific for a particular *Plasmodium ssp.*, to enable the user to distinguish between different species of the parasite in much the same way as for RFLP analysis.

The technique of AMD facilitates, not only the detection of a *Plasmodium*
10 extrachromosomal genetic element in a biological sample, but also the determination of nucleotide sequence variants which differ from the *Plasmodium* extrachromosomal genetic element probe used in the assay format.

Wherein the detection means is AMD, the *Plasmodium* extrachromosomal genetic
15 element probe is end-labelled with a suitable reporter molecule and mixed with an excess of the amplified template molecule. The mixtures are subsequently denatured and allowed to renature to form nucleic acid "probe:template hybrid molecules" or "hybrids", such that any nucleotide sequence variation between the probe and the template molecule to which it is hybridised will disrupt base-pairing in the hybrids. These regions of mismatch are sensitive
20 to specific chemical modification using hydroxylamine (mismatched cytosine residues) or osmium tetroxide (mismatched thymidine residues), allowing subsequent cleavage of the modified site using piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis followed by standard nucleic acid hybridisation as described *supra* to detect the *Plasmodium* extrachromosomal genetic element nucleotide
25 sequences.

Those skilled in the art will be aware of the means of end-labelling a genetic probe according to the performance of the invention described in this embodiment.

According to this embodiment, the use of a single end-labelled probe allows unequivocal localisation of the sequence variation. The distance between the point(s) of sequence variation and the end-label is represented by the size of the cleavage product.

5 In an alternative embodiment of AMD, the probe is labelled at both ends with a reporter molecule, to facilitate the simultaneous analysis of both DNA strands.

Wherein the detection means is IRS-PCR, the *Plasmodium* extrachromosomal genetic element probes are selected such that they each include one highly-repetitive restriction
10 enzyme cleavage site, for example *AluI*, which is ubiquitous in many genomes. According to this embodiment, the appropriate restriction enzyme cleavage site is selected such that it is ubiquitous in *Plasmodium* extrachromosomal genetic element nucleotide sequences. The amplified template DNA is electrophoresed under conditions which facilitate high resolution and optionally probed with a labelled *Plasmodium* extrachromosomal genetic element probe.
15

Optionally, the amplified template DNA may be end-filled using Klenow fragment of DNA polymerase I or other suitable means, prior to the electrophoresis step.

According to this embodiment, different combinations of probes produce different
20 patterns of amplified template nucleic acid.

Furthermore, with any probe combination used, each *Plasmodium ssp.* will produce a distinctive pattern of amplified template nucleic acid. As a consequence, the detection means is suitable for distinguishing between different *Plasmodium ssp.*, in addition to being useful
25 for the detection of the *Plasmodium* extrachromosomal genetic element *per se* in a biological sample.

Wherein the detection means is RT-PCR, the nucleic acid sample comprises an RNA molecule which is a transcription product of the *Plasmodium* extrachromosomal genetic
30 element DNA or a homologue, analogue or derivative thereof. As a consequence, this assay

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format is particularly useful when it is desirable to determine expression of one or more *Plasmodium* extrachromosomal genetic element genes.

According to this embodiment, the RNA sample is reverse-transcribed to produce the
5 complementary single-stranded DNA which is subsequently amplified using standard procedures.

The present invention clearly extends to the use of any and all detection means referred to *supra* for the purposes of diagnosing *Plasmodium* infection in humans and other
10 animals. Variations of the embodiments described herein are described in detail by McPherson *et al.* (1991), which is incorporated in the references.

A further aspect of the present invention contemplates a kit for convenient detection of a *Plasmodium ssp.* in a biological sample.

15

In an alternative embodiment, the kit of the present invention is also useful for convenient assay of infection by a *Plasmodium ssp.* parasite, wherein the sample being tested is derived from a human or other animal or mosquito suspected of being infected with said parasite.

20

The kit of the present invention is compartmentalized to contain in a first compartment, one or more nucleic acid molecules which comprise a sequence of nucleotides corresponding to a *Plasmodium* extrachromosomal genetic element or a complementary nucleotide sequence or a homologue, analogue or derivative thereof as hereinbefore defined.

25

In a particularly preferred embodiment, the first compartment is adapted to contain one or more nucleic acid molecules which are at least 95% identical to the nucleotide sequence set forth in any one or more of SEQ ID Nos: 1-4 or any one or more of the *Plasmodium vivax* or *Plasmodium malariae* sequences set forth in Figure 9 and/or Figure 10 or its complement or
30 a derivative, homologue or analogue thereof.

The kit optionally comprises several second containers comprising a reaction buffer suitable for use in one or more of the detection means described herein and optionally several third containers comprising a nucleic acid molecule positive standard, to which the assay sample result may be compared.

5

In an exemplified use of the subject kit, a negative control reaction is carried out in which the contents of the first container are contacted with the contents of the second container. At the same time, the sample to be tested is contacted with the contents of the first and second containers for a time and under conditions sufficient for hybridisation to occur. If the reagents
10 contained in the first container provided are not labelled with a reporter molecule, then the contents of the first container may be so labelled prior to the hybridisation reaction being carried out. The hybridised test sample and the negative control sample are then subjected to a detecting means as hereinbefore described. In analysing the results obtained using said kit, the control negative control reaction, test sample and nucleic acid molecule positive standard are
15 compared side-by-side. The contents of the third container should always provide a positive result upon which to compare the results obtained for the negative control and test sample. If the results of the test sample are identical to the results obtained for the negative control, then the biological sample does not contain a *Plasmodium* *ssp.* extrachromosomal genetic element. However, if the test sample produces a nucleic acid molecule which is similar or the same as
20 that contained in the positive standard, albeit of different intensity, then the biological sample contains a *Plasmodium* *ssp.* extrachromosomal genetic element.

A further aspect of the invention provides a specific extrachromosomal genetic element probe, derived from *Plasmodium* *ssp.*, or a homologue, analogue or derivative thereof,
25 according to the embodiments described herein.

In a preferred embodiment, the extrachromosomal genetic element probe is derived from a species of *Plasmodium* other than *P. falciparum*. More preferably, the extrachromosomal genetic element probe is derived from a *Plasmodium* *ssp.* selected from
30 the list comprising *P. vivax*, *P. malariae*, *P. ovale*, *P. cynomolgi*, *P. gonderi*, *P.*

(*Hepatocytis*) *kochi*, *P. inui*, *P. knowlesi*, *P. reichenowi*, *P. rodhaini*, *P. schwetzi*, *P. cathemerium*, *P. elongatum*, *P. relictum*, *P. lophurae*, *P. gallinaceum*, *P. yoelii*, or *P. berghei*, amongst others.

5 In a particularly preferred embodiment, the extrachromosomal genetic element probe is derived from *P. berghei*, *P. vivax* or *P. malariae*. More particularly, the extrachromosomal genetic element probe comprises a sequence of nucleotides which is at least 95% identical to the sequence set forth in any one or more of SEQ ID Nos: 1-15, or any one or more of the *P. vivax* or *P. malariae* sequences set forth in Figure 9 or any one or more of the sequences
10 set forth or a complementary nucleotide sequence, homologue, analogue or derivative thereof which is at least useful as a probe for the diagnosis of infection of a human or animal subject by a *Plasmodium ssp.* Alternatively, the probe at least comprises a nucleotide sequence or is complementary to a sequence which is capable of encoding an amino acid sequence set forth in Figure 11 or a homologue, analogue or derivative thereof.

15

The present invention further contemplates a kit which at least comprises one or more of the probe sequences as set forth in any one of SEQ ID Nos: 5-16.

In a particularly preferred embodiment, the subject kit comprises a first primer and
20 a second primer for the amplification of nucleic acid derived from or related to a *Plasmodium* extrachromosomal genetic element, such as a mitochondrion or plastid-like element. According to this embodiment, the first primer preferably comprises a sequence selected from SEQ ID Nos: 5, 7, 9, 12, 13, 14 or 15 and the second primer preferably comprises a sequence selected from SEQ ID Nos: 6, 8, 10 or 11 or a derivative thereof.

25

In a more particularly preferred embodiment, the first and second primers comprise the sequences set forth in SEQ ID Nos: 5 and 6, or SEQ ID Nos: 11 and 12 or SEQ ID Nos: 11 and 13 or SEQ ID Nos: 11 and 14 or SEQ ID Nos: 11 and 15.

30

The invention clearly extends to kits at least comprising one or more pairs of primus

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selected from the list comprising at least SEQ ID Nos: 5 and 6 and/or SEQ ID Nos: 7 and 8 and/or SEQ ID Nos: 9 and 10 and/or SEQ ID Nos: 11 and 12 and/or SEQ ID Nos: 11 and 13 or SEQ ID Nos: 11 and 14 or SEQ ID Nos: 11 and 15.

- 5 The invention extends further to such kits wherein both primers of a primer pair are provided in the same compartment, in aqueous solution or dried, such that the subject primers are at a relative concentration suitable for subsequent use in an amplification reaction.

 In an exemplified use of such kits, the primers are employed essentially as described
10 herein.

 The present invention is further described by the following non-limiting Figures and Examples.

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In the Figures:

Figure 1 is a copy of a photographic representation of an electron micrograph of the *Plasmodium berghei* extrachromosomal plastid preparation. P denotes the *P.berghei* plastid while M is pBR322 (4.36 kb) used as a size marker. The bar represent the size of 1 kb.

Figure 2 is a copy of a photographic representation showing the *EcoRI* and *HindIII* restriction digests of *Plasmodium berghei* extrachromosomal plastid DNA. In panel (a), plastid DNA was digested with 20 units of *EcoRI* (New England Biolabs (NEB), Beverly, MA, USA) in a reaction mixture of 10 uL. The digested products were separated on a 0.4 % (w/v) agarose gel at 120 V for 6 hours. Lane 1 shows Lambda Monocut markers (NEB, USA); lane 2 the *EcoRI* digest resulting in three fragments (E1, E2 and E3); lane 3 shows Lambda DNA-*Hind III* digest markers (NEB, USA); and lane 4 shows Lambda DNA-*BstEII* digest markers (NEB, USA). Panel (b) shows a *HindIII* digest of the extrachromosomal element of *P.berghei*. The digested products were separated on a 0.6 % (w/v) agarose gel at 100 V for 6 hours. Lane 1 shows Lambda DNA-*BstEII* digest markers (NEB, USA); lane 2 shows a *HindIII* digest of *P. berghei* DNA resulting in six fragments (H1, H2, H3, H4, H5 and H6); and lane 3 shows Lambda DNA-*BstEII* digest markers (NEB, USA).

Figure 3 is a copy of a photographic representation of a Southern Hybridization of *HindIII* and *HindIII /EcoRI* digests of the *Plasmodium berghei* extrachromosomal element. Panel (a) shows restriction digests of *P. berghei* extrachromosomal DNA. Lane M1 shows the 123 bp DNA marker (Gibco-BRL); lane HE the *HindIII /EcoRI* digest resulting in 8 fragments (H2, H3, H4, HE1, H5, HE2, E1 and E2); lane H the *HindIII* digest resulting in 6 fragments, H2, H3, H4, H5 and H6); lane M2 the Lambda DNA-*HindIII* digest markers (NEB, USA); and lane M3 the Lambda DNA-*BstEII* digest markers (NEB, USA). Panel (b) shows a Southern hybridization of the fragments in panel (a) with probe PS 1. Panel (c) shows a Southern hybridization of the fragments in panel (a) with probe PL470. Panel (d) shows a Southern hybridization of the fragments in panel (a) with probe PWQ.

30

Figure 4 is a representation of the physical and genetic map of the *Plasmodium berghei* circle. Panel (a) is a schematic representation of the arrangement of various genes and the *EcoRI* and *HindIII* sites are shown. The three *EcoRI* fragments, E1, E2 and E3 as well as the *HindIII* fragments H1, H2a, H2b as well as H4 are shown. Fragment H6 comprises of HE1, E1, E2 and HE2. The relative position of the various PCR products (Table 1) is also indicated as solid bars. Panel (b) shows a comparison between homologous genes on the *Plasmodium falciparum* and *Plasmodium berghei* plastid circles and tRNA genes are specified by a single letter amino-acid code.

Figure 5 is a copy of a photographic representation showing RT-PCR analysis of *rRNAs* transcripts. Lane 1 and 6 show the 100bp DNA ladder (Promega), lanes 2 and 3 show the RT-PCR product (L) using a set of *lsu-rRNA* gene specific primers and lanes 4 and 5 show the product (S) using a set of *ssu-rRNA* gene specific primers respectively. The (-) lanes show reactions without the reverse transcriptase enzyme.

15

Figure 6 is a copy of a photographic representation showing PCR amplification products generated using the primer set L1/L2 (SEQ ID NO: 5/SEQ ID NO: 6) (Panel a), and the primer set DHFR1/DHFR2 (Panel b). Blood was drawn daily for 5 days from a mouse initially infected with 5×10^4 parasites. Lanes 1-5 in both panels show the amplification products obtained from blood spots 1 to 5 days post-infection correspondingly. Lane 6 is the negative control with blood from an uninfected mouse and lane 7 is the positive control using 50ng of purified *P.berghei* total DNA as template. M indicates the 100bp DNA ladder (Promega) used as markers.

Figure 7 is a copy of a photographic representation showing PCR amplification of blood spots from Laotian patients diagnosed positive for *P.falciparum* malaria by Giemsa microscopy and ParaF dipstick, with the exception of one which was infected with *P. vivax* (lane 11). Primers used were the L1/L2 primer set (i.e. SEQ ID NO: 5/SEQ ID NO: 6). Lane 12 is the negative control with a blood spot from a healthy person and lane 13 is the positive control using 50ng of purified *P.falciparum* (FC27 strain) total DNA as template.

30

M indicates the 100bp DNA ladder (Promega) used as markers.

Figure 8 is a copy of a photographic representation showing PCR amplification of blood spots from uninfected persons using the L1/L2 (SEQ ID NO: 5/SEQ ID NO: 6) primer set (Panel a) and AC1/AC2 primer set (Panel b). Lanes 1 and 2 are positive controls for human β -actin using 50ng of purified total DNA from CaSki and HeLa cells. Lanes 3-10 use blood spots from uninfected persons as the template. Lanes 11 and 12 in (a) use blood spots from a *P.falciparum*-infected patient and a *P.vivax*-infected patient respectively. M indicates the 100bp DNA ladder (Promega) used as markers.

10

Figure 9 is a copy of a schematic representation of the aligned LSU-rRNA sequences from different *Plasmodium* species obtained from various regions in Asia. The alignment was carried out using the Clustal Method in the DNASTAR programme. Sequences indicated are derived from several isolates of *P.falciparum* (Pf), *P.vivax* (Pv), *P.malariae* (Pm), *P. Ovale* (Po) or *P.berghei* (Pb). The alphanumeric designation following the *Plasmodium* species descriptor indicates the isolate number and geographical origin of the specimen, wherein P=Pakistan, I=India, L=Laos, C=Columbia and S=Singapore. The GenBank accession numbers for Pf(C10) and Pb(ANKA) are X95275 and U79731 respectively. Boxes indicate residues which differ from the C10 sequence (basepair 4110-4704).

20

Figure 10 is a schematic representation of the aligned cox I sequences from *P.falciparum* and *P.vivax*. The alignment was carried out using the Clustal Method in the DNASTAR programme. Sequences indicated are derived from four *P.vivax* isolates. The GenBank accession number for the *P.falciparum* sequences is M76611. Boxes indicate residues which differ from that of M76611.

25

Figure 11 is a copy of a photographic representation showing PCR amplification of blood spots. Each reaction uses 1 μ l of blood containing different quantity of parasites. Numbers below the lanes indicate the quantity of parasites used for the PCR reactions. The detection limit is 4 parasites. M indicates the 100bp DNA ladder (Promega) used as markers.

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EXAMPLE 1

Preparation of *Plasmodium berghei* extrachromosomal DNA

Plasmodium berghei (ANKA strain) was maintained in Swiss White mice by continuous blood passage. Development of parasitemia was monitored daily by thin blood film analysis (Shute, 1988). Parasites were obtained by lysis of infected red blood cells with 1 % saponin. The extrachromosomal element was purified from the parasites using a modified procedure of the Qiagen plasmid mini preparation kit (Qiagen Inc., Chatsworths, CA, USA). Parasites from 10 infected mice (20-25 g) with a parasitemia of 60% were resuspended in 5 ml of P1 buffer, lysed with 5 ml of P2 buffer and neutralised with 5 ml of P3 buffer. After chilling on ice for 20 minutes, the precipitate was removed by centrifugation according to the manufacturer's recommendation and 200 μ l of proteinase K (50 mg/ml) were added to the supernatant, which was then reincubated for 2 hours at 37 C. The supernatant was subsequently passed through a tip-20 Qiagen column which had previously been equilibrated with 1 ml of QBT buffer. The column was washed four times with 1 ml of QC buffer each. Finally, the extrachromosomal element was eluted with 1 ml of QF - buffer which was preheated to 65 C. The DNA was precipitated with isopropanol, washed with 70% ethanol, dried and dissolved in 25 μ L of TE buffer.

20

EXAMPLE 2

Preparation and restriction digest of *P. berghei* extrachromosomal DNA

Plasmodium berghei extrachromosomal DNA was extracted from the parasite using the Qiagen plasmid mini preparation kit (Qiagen Inc., Chatsworths, CA, USA). Electron microscopic analysis of this preparation showed circular DNA elements of about 10 times the size of control pBR 322 plasmids (Figure 1). The preparation was not homogenous and, in addition to the circular elements many linear molecules of different lengths were observed. The preparation is enriched for the extrachromosomal DNA elements of both circular and linear DNA representing the homologues of the 35 kb circle and 6 kb mitochondrial DNA.

30

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These are likely to be 6 kb DNA molecules which are tandemly arrayed in head-to-tail configurations.

The extracted plastid DNA was digested into 3 fragments of 15 kb (E3), 10 kb (E2) and 5.7 kb (E1) by *EcoRI* (Figure 2a). This gives the plastid an estimated size of 31 kb. *Hind* III digest of the DNA yielded 6 fragments of 22.4 kb (H6), 4.4 kb (H5), 1.85 kb (H4), 1.23 kb (H3), 0.95 kb (H2) and 0.7 kb (H1), respectively (Figure 2b). H3 and H5 are fragments from the mitochondrial 6 kb genome.

10 The estimated size of the *P. berghei* circle is 31 kb according to Electron microscopic measurements using pBR322 as control as well as from size calculations using the *EcoR* I restriction digest fragments.

15 Extrachromosomal circular DNA has not only been found in *Plasmodium* species but also in other parasitic protozoa such as *Babesia* and *Entamoeba* (Gozar and Bagnara, 1995; Egea and Lang-Unnasch, 1995; Sehgal et al., 1994) suggesting a common evolutionary origin of this circular DNA material (Williamson et al. 1994). By maintaining such extrachromosomal information during evolution it appears that this highly conserved and seemingly functional extrachromosomal DNA molecule is important for parasite development
20 and that knowledge of its functions will greatly aid in providing novel targets for drug development.

Our preliminary tests using an antisense oligonucleotide approach indicate that this extrachromosomal element may indeed be crucial for parasite survival.

25

EXAMPLE 3

PCR amplification and sequence analysis of plastid DNA.

In order to obtain a genetic map of the approximately 35 kb *Plasmodium berghei*
30 extrachromosomal plastid, polymerase chain reaction (PCR) amplifications and sequence



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analysis of plastid DNA were carried out.

PCR was performed using the United States Biochemical (Amersham) PCR kit in a 100 μ l reaction mixture containing 2 mM $MgCl_2$, 0.2 mM of each dNTP, 4 ng/ μ L of each primer, 5 units of *Taq* DNA polymerase, 10 μ L of the 10 X PCR buffer and 1 μ L of the extrachromosomal DNA prepared as described in Examples 1 and 2. A "hot start" was carried out at 95°C for 5 minutes without the dNTPs and *Taq* polymerase. This was followed by the addition of dNTPs and *Taq* polymerase and 40 cycles of denaturation (90°C, 1.5 minutes), annealing (55°C, 3 minutes) and extension (72°C, 5 minutes). A final extension was performed at 72°C for 10 minutes.

The PCR products were loaded onto a 1 % low melting point agarose gel, extracted by the freeze-thaw method (Shoemaker and Salyers, 1990) and then cloned into the Promega pGEM-T vector.

Clone H2a was constructed by cloning the second fragment of a *Hind*III digest of the extrachromosomal element into the pBluescript vector. (Stratagene, USA).

The clones were sequenced using the ABI PRISM Dye terminator cycle sequencing kit from Perkin-Elmer on the 373A DNA sequencer from Applied Biosystems. The percentage homology with the *P. falciparum* extrachromosomal element (Accession No. X 95275 and X 95276) was obtained using the Martinez/Needleman-Wunsch DNA alignment programme from DNASTAR.

PCR amplification of different parts of the extrachromosomal plastid were performed using primer sets homologous to sequences from the 35 kb circle of *P. falciparum* (Table 1). These include the primer sets comprising SEQ ID Nos: 5 and 6 (L/L Primer set), SEQ ID Nos: 7 and 8 (L/S primer pair) and SEQ ID Nos: 9 and 10 (S/S Primer pair, homologous to the small-subunit (ssu) -*rRNA* of *P. falciparum*).

30

The amplified regions obtained with these primers lay within the large subunit (*lsu*) *-rRNA* gene, *rpo B* gene, the cluster of 10 *tRNAs*, part of the cluster of four *tRNAs* located close to the 3' end of the *tufA* gene in *P. falciparum* as well as the region between the *lsu-rRNA* and the *ssu-rRNA* genes.

5

All PCR fragments were cloned into the pGem-T vector from Promega. Sequence analysis performed using the Martinez/Needleman-Wunsch DNA alignment on all clones which had been purified using Qiagen midi plasmid preparation columns showed a similarity index of greater than 80% with the *P. falciparum* circle except for the PPH and PWQ
10 fragment (Table 1). The PRB fragment was homologous to the *P. falciparum rpo B* gene with a similarity index of 87.9% for the DNA sequence and 85.6% for the corresponding amino acid sequence (using the Lipman-Pearson protein alignment). The PPH sequence spanning the cluster of 10 *tRNA* genes had a similarity index of only 78%. While the *tRNA* coding regions were highly similar to those in *P. falciparum* the non-coding spaces were much less conserved
15 between the two Plasmodium species.

In order to examine if the *lsu-rRNA* gene in *P. berghei* exists as a repeat, a single forward primer (L3) homologous to the 3' end of the *P. falciparum lsu-rRNA* sequence and 2 distinct reverse primers homologous to the ORF 470 (04) and the start of the cluster of 10
20 *tRNA* genes (3H) of the *P. falciparum* circle were designed. The fragment amplified with the L3/04 primer set (PL470) was distinct from that amplified using the L3/3H set (PLH). Sequence analysis of PL470 showed a homology of 83% with the same region in *P. falciparum*. The sequence of PLH was homologous to the 3' end of the *lsu-rRNA* and the 3' end of *rps 4* of the *P. falciparum* circle (data not shown) indicating that the *lsu-rRNA* gene
25 exists as a repeat in *P. berghei*. In addition, a cluster of intervening *tRNA* genes was present between the *lsu-rRNA* and *ssu-rRNA* genes (fragment PLS). This repetition and arrangement is similar to the organisation of the *P. falciparum* circle, where a palindromic sequence of genes for the subunit *rRNAs* and several *tRNAs* exists. Each arm of the palindrome contains one *ssu* and one *lsu-rRNA* gene and a cluster of intervening *tRNA* genes (Gardner *et al.*,
30 1993).



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From the nucleotide sequences set forth in SEQ ID Nos: 1-4, it is clear that the genes in the *P.berghei* circle are homologous to those in the 35 kb *P.falciparum* circle. Major differences in sequence are observed in the non-coding spaces between tRNA gene clusters. The arrangement of genes appears to be similar in both Plasmodium species and a repeat of 5 the rRNA genes does not only exist in *P.falciparum* but also in the *P.berghei* circle.

EXAMPLE 4

Southern Hybridisation of restriction fragments

10

Fragments from the *Hind*III and *Hind*III/ *Eco*RI digests of the *Plasmodium berghei* extrachromosomal element were separated on a 1 % (w/v) agarose gel at 120 V for 4 hours. The separated fragments were then transferred onto a Nylon membrane (Hybond-N, Amersham) by capillary action using 20x SSC buffer (0.3M sodium citrate, 3M sodium 15 chloride, pH 7.0). Southern hybridization was performed using probes specific for the approximately 35 kb circle that were made from the cloned PCR products, according to the preceding Examples.

The PCR products were liberated from the vector by digestion with *Apa*I and *Pst*I. The 20 enhanced chemiluminescence (ECL) direct nucleic acid labelling and detection system (Amersham International PLC, England) was used for labelling the probe, for hybridisation and for detection.

Table 1. Description of clones of various segments from the extrachromosomal element in *P. berghei* and their percentage homology with *P. falciparum*.

5	Name of clone	Description	Size (bp)	Percentage homology with <i>P. falciparum</i>	<i>EcoRI/HindIII</i> sites
	H2a	Second fragment of <i>HindIII</i> digest containing SSU rRNA	949	92.3	Two <i>HindIII</i> sites
	PS1	PCR product of SSU rRNA	526	94.3	Nil
	PL1	PCR product of LSU rRNA	595	95.5	One <i>HindIII</i> site
	PL2	PCR product of LSU rRNA	595	93.8	One <i>HindIII</i> site
10	PL3	PCR product of LSU rRNA	735	88.8	One <i>HindIII</i> site
	PLS	PCR product of tRNAs between LSU and SSU rRNA	973	87.3	Nil
	PPH	PCR product of tRNAs before the repeat	1000	78.0	One <i>EcoRI</i> site
	PLH	PCR product from LSU rRNA to His-tRNA	1118	82.3	Nil
	PL470	PCR product from LSU to ORF470	1125	83.0	Nil
15	PRB	PCR product of the RpoB gene	516	87.9	One <i>EcoRI</i> site
	PWQ	PCR product of the Phe-tRNA	161	69.6	Nil
	PB-1	Sequence derived from clones spanning the Ile-tRNA, the ssu-rRNA, the lsu-rRNA and the ORF-470 genes	5849	88.5	4 <i>Hind III</i> sites
	PB-2	Sequence derives from clones spanning the regions within the lsu-rRNA, the rps 4 and the cluster of 10 tRNA genes	2621	80.2	1 <i>Hind III</i> site and 1 <i>Eco RI</i> site



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Each of these steps were carried out according to the manufacturer's instruction. First, 8 μ g of probe in a volume of 20 μ L were denatured by boiling for 5 minutes and immediately cooled on ice for 5 minutes. 20 μ L of labelling reagent were then added. This was followed by the addition of 20 μ L of glutaraldehyde solution. The mixture was incubated for 20 minutes at 37°C before addition to the hybridization buffer. The ECL Gold hybridisation buffer containing 0.5M NaCl and 5% blocking agent was used for hybridisation. The blots were prehybridised for 2 hours at 42°C and the labelled probe was added to a final concentration of 800 ng DNA/ml. Hybridisation was allowed to proceed overnight at 42°C. The blots were washed twice in primary wash buffer containing 6M urea, 0.4% SDS and 0.5x SSC at 42°C for 20 minutes. This was followed by two rounds of washing in 2x SSC buffer at room temperature for 10 minutes. For detection, 6.5 ml of equal volumes of detection reagents 1 and 2 were mixed and added to the blot for 1 minute. The blot was then drained, wrapped in Saran Wrap and the DNA side was exposed to an autoradiography film.

A double digest of the *P. berghei* circle with *Hind*III followed by *Eco*RI resulted in the following fragments: 10kb (E2), 5.7kb (E1), 5.0kb (HE2), 4.4kb (H5), 2.3kb (HE1), 1.85 (H4), 1.23 (H3), 0.95(H2) and 0.7kb (H1). The PS 1 probe hybridised to H2, the PL470. probe hybridised to H6 and HE2 while the PWQ probe hybridised to H6 and E2 (Fig. 3).

20

The results obtained with various other probes are shown in Table 2. Of interest to note is that H2 contained 2 distinct fragments which hybridised with PS 1 and PL3. One of the H2 fragments (H2a) was cloned into Bluescript vector (pBS KS (II)+) and sequenced. The sequence corresponded to the internal region of the *P. falciparum* 35 kb ssu-rRNA gene (Table 1, sequence H2a). The other fragment (H2b) arose from the two *Hind* III sites within the lsu-rRNA gene. The presence of these two sites was confirmed by the sequences from the PL2 and PL3 PCR products. In addition, both the H5 and the H3 fragments hybridised with probes corresponding to the *P. berghei* 6 kb mitochondrial DNA. This was not unexpected as the preparation was found to contain linear molecules as shown by electron microscopy (Fig. 1).

30

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Table 2. Southern analysis of restriction digests.

5	Probe	<i>Hind</i> III digest	<i>Hind</i> III/ <i>Eco</i> RI digest	<i>Eco</i> RI digest
10	PS1	H2	H2	N.D.
	PLS	H4	H4	N.D.
	PWQ	H6	E2	E2
15	PL1	H6	HE2	N.D.
	PL2	H6	HE1, HE2	N.D.
	PL3	H2, H4	H2, H4	E3
20	PRB	H6	HE2	E2, E3
	PL470	H6	HE2	E3
	PPH	H6	HE1	E3
25	PB6K-4	H3, H5	H3, H5	N.D.
30				

EXAMPLE 5

Physical and genetic map of the *P.berghei* 35 kb circle

A map of the approximately 35 kb *P. berghei* extrachromosomal circle was constructed
 5 based on the information from the restriction digests, Southern hybridisation experiments and
 the sequence analysis of the PCR fragments (Figure 4a). The PPH and PRB fragments each
 contained an *EcoR* I restriction site (Table 1). The three *EcoR* I and six *Hind* III fragments
 were arranged according to their hybridisation patterns. The PRB probe hybridised to both
 the E2 and E3 fragments from the *EcoR* I digest indicating that E2 is positioned next to E3
 10 (Table 2).

The HE2 fragment obtained from the double digest with *EcoR* I and *Hind* III,
 hybridised to probes PRB, PL470 and PL2 while H2b and H4 hybridised to probe PL3. Both
 the PL2 and PL3 fragments are regions within the *lsu-rRNA* gene, whereas the PL470
 15 fragment contains 3' end of the *lsu-rRNA* gene. Thus, the ORF470 must be located next to
 the PL2 fragment. H2b is situated between HE2 and H4 since H4 also hybridised with the
 PLS probe which contains the 5' ends of both the small and large subunits rRNA genes. H2a
 hybridised with probe PS 1 which corresponds to a region within the *ssu-rRNA* gene,
 therefore H2a must be located next to H4. Finally, HE1 is placed next to E1 as HE1
 20 hybridised to probes PPH, PLH and PL2.

The arrangement of genes on the *P. berghei* circle spanning the *rpo B* gene and the
 cluster of 10 *tRNAs* genes is thus very similar to that of the *P.falciparum* 35 kb circle (Figure
 4b). The *P.berghei* circle encodes organelle-like rRNAs, tRNAs, ribosomal proteins and
 25 RNA polymerase subunits, similar to those identified for *P. falciparum* (Preiser *et al.*, 1995).

EXAMPLE 6

Reverse transcription-PCR of LSU-rRNA and SSU-rRNA

In order to determine if the approximately 35 kb *Plasmodium berghei* extrachromosomal genetic element is transcriptionally active, total RNA from *P. berghei* was isolated using the RNeasy total RNA kit (Qiagen Inc., Chatsworth, CA, USA) and a combined reverse transcription - PCR (RT-PCR) reaction was carried out to amplify *lsu-rRNA* or *ssu-rRNA* transcripts.

Total RNA was isolated from *Plasmodium berghei* using the Qiagen RNeasy Total RNA kit (Qiagen Inc., Chatsworth, CA, USA). Parasites from 10 infected mice with a parasitemia of 60% were resuspended in 350 μ l of lysis buffer RLT and homogenised using a QIAshredder (Qiagen Inc.). The homogenate was cleared of insoluble material by centrifugation and 1 volume of 70% ethanol was added. The entire sample was then added to the RNeasy spin column and washed with RW1 buffer followed by two washes with RPE buffer. The RNA was eluted out with 35 μ l of water. 5 μ l of the RNA was used as starting material for the Access RT-PCR system (Promega, Madison, USA). Two primer sets, L/L (SEQ ID Nos: 5 and 6) and S/S (SEQ ID Nos: 9 and 10) were used. The manufacturer's protocol was followed with the exception of the annealing step for PCR amplification. Annealing was allowed to proceed at 55°C for 1 minute. The PCR products were separated on a 1% (w/v) agarose gel and visualised by ethidium bromide staining.

Amplification using the RT- PCR kit from Promega and a set of primers homologous to the *ssu-rRNA* produced a 526 bp fragment while amplification using a set of *lsu-rRNA* specific primers resulted in a 594bp fragment (Fig. 5).

EXAMPLE 7**Assay of blood samples for the presence of *Plasmodium* spp.**

A total of 482 *Plasmodium*-infected blood samples from four different locations,
 5 Singapore, Laos, Pakistan, India and Colombia and a defined number of negative control
 blood samples, were analysed for the presence of *Plasmodium* extrachromosomal genetic
 elements, using the polymerase chain reaction.

Briefly, 10-100 μ L of whole patient blood (either peripheral blood from a finger prick
 10 sample or venal blood) was spotted onto a filter disc or equivalent solid support and directly
 amplified, using each of the primer pairs:

L/L PRIMER PAIR:

	SEQ ID NO:5:	5'-GACCTGCATGAAAGATG-3'
15	SEQ ID NO:6:	5'-GTATCGCTTTAATAGGCG-3'

L/S PRIMER PAIR:

	SEQ ID NO:7:	5'-GCCACTACTATGAAAATC-3'
	SEQ ID NO:8:	5'-GCGTTCATTCTGAGCTAG-3'

20

S/S PRIMER PAIR:

	SEQ ID NO:9:	5'-GCGGTAATACAGAAAATGCAAGCG-3'
	SEQ ID NO:10:	5'-AGCACGAACTGACGACAGCCATGCAC-3'

25 PCR Buffer used in the amplification reactions comprised the following:

	70mM Tris. pH8.8
	20mM Ammonium sulphate
	1 mM DTT
	0.1 μ g/ μ L BSA (or 0.01 % geletin)
30	2.5mM MgCl ₂

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Each 100 μ L reaction included 0.4 μ g of each primer, 0.8mM dNTP mixture and 5U of *TaqI* polymerase.

The template DNA was fixed with methanol for 5 mins. A "hot start" was carried out at 95°C for 5 minutes without the dNTPs and *Taq* polymerase. This was followed by the addition of dNTPs and *Taq* polymerase and 40 cycles of denaturation (90°C, 1.0 mins), annealing (60°C, 2 mins) and extension (72°C, 3 mins). The PCR products were analysed by agarose gel electrophoresis.

The results are shown in Tables 3 and 4. The L/L primer set was capable of identifying *Plasmodium falciparum*, *P. vivax* and *P. malariae* in 100% of cases, suggesting that this primer pair is useful in the genera-specific diagnosis of *Plasmodium* infection. The S/S primer set was capable of efficiently diagnosing *P. falciparum* and *P. malariae* in 100% of cases. In marked contrast, the L/S primer set resulted in only poor diagnosis of *P. vivax* and *P. malariae*, however detected the presence of *P. falciparum* in blood samples, suggesting that this primer pair is species-specific. The human actin primer set AC1/2 were used as positive controls.

Results also indicate that the selection of primer pairs in the diagnostic assay was of primary importance in determining the reliability of the assay in diagnosing infection by *Plasmodium ssp.*

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TABLE 3
Number and origin of *Plasmodium* infected samples

	Origin	Number	P.fal	P.viv	P.mal	P.ova	Mixed	Controls
5	Singapore	74	15	26	2	2	3	26
	Laos	16	15	1	-	-	-	-
	Pakistan	68	14	53	-	-	-	1
	India	11	1	10	-	-	-	-
10	Colombia	313	1	29	-	-	-	283
	Total	482	46	119	2	2	3	310

15 **TABLE 4**
PCR results using the primer pairs L/L, L/S, S/S and AC1/2

	Species	L/L	L/S	S/S	AC1/2
20	P.fal	46/46 (100%)	14/20 (70%)	21/21 (100%)	nd
	P.viv	119/119 (100%)	6/58 (10%)	48/57 (84%)	nd
	P.mal	2/2 (100%)	0/1 (0%)	1/1 (100%)	nd
	P.ova	2/2 (100%)	nd	nd	nd
	mixed	3/3 (100%)	nd	nd	nd
25	controls	0/310 (0%)	nd	nd	92/92 (100%)

nd = not done

30

EXAMPLE 8

Direct PCR amplification of extrachromosomal *Plasmodium*

DNA from dried blood spots

5 1. *Specimen Collection*

Blood was collected by fingerprick (5-10 μ l) or by venipuncture from subjects with Giemsa smear-positive *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* malaria as well as from healthy controls, and spotted in replicates onto Whatman filter paper. *Plasmodium berghei* (ANKA) infected mouse blood (5 μ l) was collected from the tail.

10 *Plasmodium berghei* infections were maintained by serial blood passage of 10⁷ parasites. Dried blood spots were placed individually into 200 μ l PCR tubes and fixed with the addition of methanol for 5 minutes. The methanol was poured off and the blood spot was dried thoroughly prior to PCR amplification.

15 2. *PCR amplification*

Amplification was carried out as previously described (Long *et al*, 1995) with some modifications. Each 100 μ l reaction mixture contained 1xPCR buffer (70 mM Tris, pH 8.8, 20 mM (NH₄)₂SO₄, 1 mM DTT, 0.1 μ g/ μ l BSA) 2.5 mM MgCl₂, 0.4 μ g of each primer, 5 units of Taq DNA polymerase (Amersham) and 0.2 mM of each dNTPs. Reaction tubes were

20 overlaid with one drop of mineral oil. The reaction was soaked at 95°C for 5 minutes then held at 80°C prior to the addition of Taq DNA polymerase and dNTPs. Amplification involved 40 cycles of 1 minute denaturation at 90°C, 2 minute annealing at 52°C and 3 minutes primer extension at 72°C. A 5 minute primer extension at 72°C was included following the final cycle.

25

3. *Sequences of primers.*

Three sets of primers were used in this study. The primers used for amplifying the LSU-rRNA gene were L1 5' GAC CTG CAT GAA AGA TG3' (SEQ ID NO: 5) and L2 5'GTA TCG CTT TAA TAG GCG3' (SEQ ID NO: 6). A second set of primers designated

30 as DHFR1 (5'GCA ATA TGT GCA TGT TGT AAA3') and DHFR2 (5'ATT CTT TAT

AAA CAG ACG3') were designed to amplify the dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene from *P.berghei* genomic DNA in control experiments. The primers used for amplifying the human β -actin gene were AC1 (5'GGG CGA CGA GGC CCA GAG C3') and AC2 (5'GCA TCC TGT CGG CAA TGC C3').

5

4. *Agarose gel electrophoresis*

10 μ l of each PCR product was resolved in 1% agarose gels with TAE electrophoresis buffer (40mM Tris-acetate and 1mM EDTA, pH 8.0). Electrophoresis was carried out at 100V for 1.5 hours and the fragments were visualized under UV.

10

5. *DNA sequencing protocol*

The PCR products were loaded onto a 1% (w/v) low-melting point agarose gel and extracted by the freeze-thaw method (Shoemaker and Salyers, 1990). They were then cloned into the pGEM-T vector (Promega). The clones were sequenced using the ABI PRISM Dye
15 terminator cycle sequencing kit (Perkin Elmer) on the 373 DNA sequencer from Applied Biosystems. Multiple sequence alignment using the cluster method was carried out with the DNASTAR programme.

6. *Results*

20 6.1 *Detection of P.berghei infection in blood spots*

Conditions for the PCR amplification of *P.berghei* infected mouse blood spotted on filter paper were optimised using DHFR1 and DHFR2 primers. Once these conditions were established, the sensitivity of the LSU-rRNA primer set was compared with that of the DHFR-TS primer set. The LSU-rRNA primer set was designed to amplify a 594bp fragment
25 from the *P.berghei* circular DNA while the DHFR-TS primer set amplified a 511bp fragment from *P.berghei* genomic DNA. Blood spots were prepared daily for 5 days from a mouse which was initially infected with 5×10^4 parasites. Giemsa staining of thin blood films from the same animal was done daily. The LSU-rRNA primer set was more sensitive than the DHFR-TS primer set in detecting parasite DNA. The amplified LSU-rRNA fragment was
30 detectable by ethidium bromide staining one day after infection (Figure 6a) while the DHFR-

TS PCR product was only visible two days post-infection (Figure 6b). At these two time points, no parasite was detected on the corresponding Giemsa-stained blood films. Parasites were only observed on the film three days post-infection.

5 6.2 PCR amplification of blood spots from malaria infected patients.

The above PCR amplification protocol was also applied to blood spots from 31 malaria-infected patients. 15 of these samples were obtained from patients admitted to the National University Hospital in Singapore. Of these, 7 had *P.falciparum* infection, 1 had *P.malariae* and the remaining had *P.vivax* as determined by Giemsa and Quantitative Buffy
 10 Coat (QBC) diagnosis. All samples were positive for amplification with LSU primers (data not shown). The other 16 samples were from patients in Laos with 15 *P.falciparum* infections and one *P.vivax* malaria infection as determined by Giemsa diagnosis. LSU-rRNA PCR amplifications were positive for all 16 specimens. As shown in Figure 7, the PCR products from 11 of the 16 Laotian specimens. Eight healthy persons and total DNA from
 15 two human carcinoma cell lines, CaSki and HeLa were used as controls. These were all negative when using the LSU-rRNA primer set for PCR amplification but were all positive for human β -actin (Figure 8, compare panels a and b).

EXAMPLE 9

20 Sequence alignment of LSU-rRNA extrachromosomal DNA from various *Plasmodium* species

The LSU-rRNA fragments amplified from the blood spots as described in Example 8 were cloned into the pGEM-T vector and sequenced. In addition to amplified products from
 25 the Singaporean and Laotian patients, we also amplified and sequenced LSU-rRNA fragments from Indian, Colombian and Pakistani patients. The published *P.falciparum* sequence (C10 strain) was used as the basis for all alignments and comparisons.

Comparison of the *Plasmodium* species used in this study showed that this region of
 30 the LSU-rRNA gene is highly conserved and the similarity between *P.falciparum*, *P.vivax*,

P. malariae, *P. ovale* and *P. berghei* is greater than 91% (Table 5). The similarity between the C10 and other *P. falciparum* sequences ranged from 98.3%-99.8%, while that between the C10 and the *P. vivax* sequences ranged from 91.1- 99.7%. The greatest divergence in sequence was observed from the *P. vivax* specimens from Pakistan and Colombia. In all 5 cases, divergence in sequence was due to 1 or 2 base changes in isolated regions within the LSU-rRNA fragment (Figure 9).

TABLE 5
Percent homology of LSU-rRNA sequences with *P. falciparum*
(C10 strain) sequence

Name of sequence ¹	Similarity Index to Pf(C10) ²
Pf10/P	98.3
Pf11/P	98.5
Pf19/I	99.7
Pf20/L	99.7
Pf18/S	99.8
Pv12/P	93.4
Pv13/P	92.9
Pv15/I	99.5
Pv16/L	99.7
Pv17/S	93.4
Pv86/C	91.1
Pm1/S	93.2
Pm38/S	92.9
Po35/S	93.4
Po36/S	93.2
Pb(ANKA)	94.2

¹ Pf denotes *P. falciparum*, Pv denotes *P. vivax*, Pm denotes *P. malariae* and Pb denotes *P. berghei*. The alphabet at the end of each name indicates the origin of the specimen; P=Pakistan, I=India, L=Laos, C=Colombian and S=Singapore. The GenBank accession numbers for Pf(C10) and Pb(ANKA) are X95275 and U79731 respectively.

² Similarity index obtained using the Martinez-Needleman-Wunsch DNA alignment programme.

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EXAMPLE 10

Discussion

In this study, we have shown that it is possible to amplify the extrachromosomal
5 circular plastid-like DNA found in *Plasmodium* spp. This has allowed us to proceed with
characterising the LSU-rRNA gene from the circular DNA of malaria-infected patients using
only a small volume of blood spotted on filter paper.

We have designed a pair of primers based on the sequences from *P.falciparum* and
10 *P.berghei* such that the primers are completely homologous for both species. Using these
primers, we have been able to amplify the corresponding LSU-rRNA fragment from
P.falciparum, *P.vivax*, *P.malariae* and *P.berghei* infected blood. Sequence analysis of these
fragments indicates that this region of the LSU-rRNA is highly conserved between different
species of *Plasmodium*. In addition, different geographic isolates of *P.falciparum* and *P.vivax*
15 from Asia do not show distinct variations for the LSU-rRNA fragment. GenBank searches
indicate that this fragment sequence is unique.

The high homology between the various *Plasmodium* species has led us to examine if
the LSU-rRNA specific primers are useful for the detection of malaria infections. Using
20 *P.berghei*, the LSU-rRNA primer set was shown to be more sensitive than the DHFR primer
set in parasite detection in mouse blood spots. All 31 patient blood spots tested were positive
regardless of the *Plasmodium* species involved while none from healthy persons was positive.
These results indicate that the LSU-rRNA primers may be useful for the diagnosis of malaria
infection.

25

The ease of direct PCR amplification of extrachromosomal *Plasmodium* circular DNA
from dried blood spots has provided us with the means to study and characterise the genes
present on this DNA molecule. To date, none of the genes on the circular DNA of *P.vivax*
and *P.malariae* has been described. This is the first description of an analysis of the LSU-
30 rRNA gene from different field isolates of *P.vivax*, *P.malariae* and *P.falciparum*. More

investigations are being carried out to determine the extent of sequence conservation and arrangement of the genes on the circular DNA from different *Plasmodium* species.

EXAMPLE 11

5 **PCR amplification and sequence analysis of cox I gene**

To obtain the complete sequence of the mitochondrial cox I gene, a set of primers was designed based on the published *P.falciparum* sequence (GenBank accession number M76611). PCR using this primer set with blood spots from *P.vivax* infected patients resulted in fragments
 10 of 1.5kb in size (Figure 10). These were cloned into pGEM-T vector (Promega). The clones were sequenced in both directions using the ABI PRISM dye terminator cycle sequencing kit on the 374 DNA sequencer from Applied Biosystems. DNA sequence alignments were carried out using the Martinez/Needleman-Wunsch DNA alignment. The DNA sequences from 4 different *P.vivax* isolates were highly conserved (greater than 99% similarity). However, these
 15 sequences were less homologous (83%) when compared with the corresponding cox I gene from *P.falciparum*.

EXAMPLE 12

Plasmodium species identification in blood samples

20

In order to differentiate between *P.vivax* and *P.falciparum* infection, one set of *P.vivax* specific primers (PV1 - SEQ ID NO 12 and P2 - SEQ ID NO 11) and three sets of *P.falciparum* specific primers (PF1 - SEQ ID NO 13 and P2 - SEQ ID NO 11, PF2 - SEQ ID NO 14 and P2 - SEQ ID NO 11, PF3 - SEQ ID NO 15 and P2 - SEQ ID NO 11) were designed based on the
 25 mitochondrial cox I genes of the two species. PCR assays were carried out on whole patient blood spotted onto a filter disc as described for example 7. The PCR products were analysed by agarose gel electrophoresis.

The results are shown in Tables 6, 7 and 8. The three sets of *P.falciparum* specific
 30 primers only detected *P.falciparum* infected blood but not the other three human *Plasmodium*

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species. The *P. vivax* specific primers (PV1/P2) detected only 92% of the *P. vivax* infected blood and a false positive with *P. malariae* was also observed. This primer set does not react with *P. falciparum* or *P. ovale* (Table 6).

5

TABLE 6

Results using Plasmodium species specific primer set PF1/P2 and PV1/P2

	Species	<i>P. falciparum</i> primer set I (PF1/P2)	<i>P. vivax</i> primer set I (PV1/P2)
10	P.fal	12/12	0/12
	P.viv	0/26	24/26
	P.mal	0/2	1/2
	P.ova	0/2	0/2
	Pm/Pf	1/1	0/1
15	Pv/Pf	2/2	2/2
	Controls	0/7	0/7

TABLE 7

20

Results using Plasmodium species specific primer set PF2/P2

	Species	<i>P. falciparum</i> primer set II (PF2/P2)
25	P.fal	6/6
	P.viv	0/14
	P.mal	0/2
	P.ova	0/2
30	Controls	0/7

TABLE 8
Results using Plasmodium species specific primer set PF3/P2

5	Species	<i>P.falciparum</i> primer set III (PF3/P2)
	P.fal	9/9
	P.viv	0/12
	P.mal	0/2
	P.ova	0/2
10	Controls	0/7

EXAMPLE 13

15 **Sensitivity of PCR assay for Plasmodium species in blood samples**

The minimum number of parasites detectable by PCR assay was determined by using 1µl of whole patient blood or diluted blood spotted on filter disc. Using the L/L (SEQ ID NOS 5 and 6), the minimum number of parasite detected is 4 (Figure 11).

20 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. The invention also includes all of the steps, features, composition and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of
25 said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(I) APPLICANT: THE NATIONAL UNIVERSITY OF SINGAPORE

(ii) TITLE OF INVENTION: DIAGNOSIS OF PARASITES

10

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

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(i) SEQUENCE CHARACTERISTICS:

- 53 -

(A) LENGTH: 5849 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10	TAATGAAGCT GTACATCCTT CTAAATATCC AACATATGCA AATTCAGTTG CTATTAATAA	60
	AGTACGTTCA AATTGTGCAA AATCATAAGA ATTAGTCTTA AAATAAGTTG ATAAATTAAA	120
	ACTACATTTT ATATACTTAG ACACATAACA AAAAGATCCT TCACTAAAAA TAATTGAATT	180
15	AATATTGCA AAAAAATTAT CTTATAAGA AACTACAGTT CCTAAATATT TTTTACTAA	240
	TAAAGGATAT TTTAAAATAA CGTCCAATAA AGACAAAAT ATAATACCTA ATTTTTTTAA	300
20	AAAATATTGT GTTGATGTA AAACAGATAT ACTATCACAA ATAACATCA TAGGAATTAT	360
	TTTTTTATTA AAATAGGTAT CTAAAAAATT TATATTTAA TTAGTTTTTA AATATACTAA	420
	CAAATTACTA TCTTTTAAAG TAGAAGAATA ATAAATAATA TTATCATAAC TAATATTGGG	480
25	ACATTGAAA CACGACCAAT CTGGTAATTT AACATATTT AAAAATTTTA AAGAATATAT	540
	TTTAAATTTG TAAATAAAAA AATATAAATA AATATTATTA GATAAATTTT TTATCAAATT	600
30	TTTATTTAAT CCATTCTTA TTAAATATAA ATTTATTTTA TTATTATATT GATATTTATA	660
	ATTTAAATTA TAAATATTTA AAAATTTTTT TAATTTTAAT TTATTTATCA TAATAATTTT	720
	ATATTATAAA ATATTTCAAG TTAACGATGA GATTTGAACT CACAATCTAC TGATTACAAA	780
35	TCAGTTGCTT TACCAATTAA GCCACTTTAA CAAATATAAT ATTTATAATT AAATATTCAA	840
	CTTATTAGGA ATTATACACA AAATATATTA CTATAAATAC ATATTAATTC TATAAAATAA	900
40	TTTTTCTAAT TATTGTTTTA TTCATTTATA TGATTAGAAT ATTATTTTTA ATTAAATTTT	960

- 54 -

	CTTATTTATA TTACTTCAAC AATTAAAATT TTATACTTAA CTA CTACTCAACA TTACAAAATA	1020
	TAATAATTGA TATATCATTG GTATAATTTT TTCGATCCTC TCGTACTAGA AAAAATAATT	1080
5	TCAATATTCT AACACTTATA TTAGATATGG ACCGAACTGT CTCACGACGT TCTGAACCCA	1140
	GCTCACGTAT CGCTTTAATA GGCGAACAGA CTTACCCTTA AACATACTA CTGCCTTAGG	1200
	ATGCGATAAG CCGACATCGA GGTGCCAAAC CTTTTCGTCA ATATGGACTC TCGGAAAAGA	1260
10	TTAGCCTGTT ATCCCTAGAG TAACTTTTAT CCGTTAAGCG ATAATTTTAT TATTAAATAA	1320
	TTATCGGATC ATTAAGACCG ACATTAATCT CTGTTTAATT TGTAATTTT ACAGTTAATT	1380
15	ATATATTTAT CTTTATATAA TAAATATAAC ATTGTACACC TCCGTTTTTA TATAGGAGGA	1440
	GACCGCCCCA GTCAAATAT CTTATAAATA TTGTTAAAAA TTTTGTATA AAAATTTTAT	1500
	AAGAATTTAT ATATATATAA AATGGTATTT CATTACAAT TACATTATTT CCAAAAAAAT	1560
20	AATATTACTA CTTCCCATTT ATTCTATGTT ATATATATAT ATTTTCAATA TCTATTAATA	1620
	GTAAAGCTTC ATAGGTCTT TCTGTCCTAA TATAAGAAAT CTGCATCTTC ACAGATAATT	1680
25	TTATTTCAAT AAGATTTTTT TTAAGACAGC ATTTAAGTCG TTACATCTTT CATGCAGGTC	1740
	GGAACCTACC CGACAAGGAA TTTCGCTACC TTTGGACCGT TATAGATACA GCCGCCGTTT	1800
	ACTATAGCTT ATATATATAT TATAATTTTA AATTATAAAT ATTATTTTTA CATAATAGCA	1860
30	CTGGGCAGAT GTCAATCTTT ATACATCATC TTTCGATTTA GCAAAGATTT GTGTTTTTGT	1920
	TAAACAGTCG CTTAAATTTT TTGTTTTCAA CTAAATAAGT ATCTCTTCTC CCCTAAGTTT	1980
35	ACGAGATAAA TTTGCCGAGT TCCTTAAAAA AAATTATCTC AACTTCTTAA TAATTTATAT	2040
	ATATTTACTA GTGTCAGTTT ACAGTACGAA TACATAATAA TATATATATA TAAATAATTT	2100
	TTATATAATA TAATATATTT ATTATTATAT TAGTTTTAAA ATATAAATAT TATTATATAG	2160
40		

- 55 -

TATAAGAATA TTAACCTATT ACCTATCGAT TACACATTAC ATCTCATCTC AAGATACGAC 2220
 TAACCCTATT TAAAATAATA ATAAATAGGA GCCCTTAAAT TATAGAAGTA TTGGATTTTT 2280
 5 ACCAATATTT ACATTACTCA AATTAGCATT ATCACTTTTG ATATAATTAT TTAACTTTT 2340
 CATATAAATA ATTTATATTC AAAACGCTCT TTTACCAATT TAATTTTATT AATATTAAAT 2400
 10 TTTATACATA TCGATAATTA ATTTATTTTC GATTATTCTT GAACTAAAAT TACTAAATTA 2460
 ATGAGCTTTT ACGCACTCTT TAAAAGATAA CTGCTTCTAA ATTTACTTTT TAATTATTTA 2520
 AATAATTTTA TATTCTTTT AAGACTTAAT TAATATTTAA AAATCTTAAT TTATAATTCC 2580
 15 GGCTGTTTCC CTTTTGAAAA TAAAGCTTAT CCTTTATTTT CTGATCATAT ATATATTTTA 2640
 TTAAATAAAA TTCTTAAATT ATTTTCATTA ATATTAATA TATAAATTAA TTTAATAAAA 2700
 AAAGAGTTTT ACATTTATTT ATATATAAAT ACTATACTTA CATATATTTT AAAGAGAACC 2760
 20 AGCTATCTTC AAATTCGATT GGCATTTTAC CTCTAATTAT ACTTTATTTG ATACTTTTGC 2820
 AACAGTAACC AATTCAAAC TCAATTTAAT TTTATTTAAA TCTTATTTTA AATATAATTA 2880
 25 GATCATTTGA TTTCGGGTCT ATAATAAATA ATATACTAAA TGCTTATTAT ATATAATAAC 2940
 AAACCTGAGT ATACTTTGGC TTCATTTATA AATATTTAAC CTAATAATTA TACTATTTAT 3000
 TATAACTTGC TAATTCCTTC TTCAACAAGA AAATAATAAA ATTATATTAA ATTTTATTAT 3060
 30 TATTTATTAA ATTTAAAATT CAGGTTCTTT TCACTATTTT CTCAAAATCC TTTTCATCTT 3120
 TCCCTCACGG TACTATTCAC TATCAACTTT TATTATATTA AATTTTATAA GATAACTCTT 3180
 35 AATTATATTT ATATTATTCA TATAAAATAT ATTTTATAT TACTTAATTA AAATTTTACA 3240
 TATATAATGT TTTAAATCTT TCAGTTTCGCT CGCCACTACT ATGAAAATCG TTATTACTTT 3300
 40 ATATTCCTTT AAGTACTAAG ATGATTCAGT TCCTTAAGTT TTTTAAAAT ATTTATATAA 3360

- 56 -

AAATAAATTT TTATTCAGAT ACTTTTATAA TTTAATAAT AAAAAATTTT AAATATATTT 3420
 AATTTTTTAT AATTATAAAA ATTCGTAA TATATTTAAC GTCTTCTTC AATAATAAAA 3480
 5 ATAATAGACA TCCTTTTAAA TTTATTATAT ATATTTAATT ATATATTTAA CTATATAAAT 3540
 TATAAATTAA TTTATTTAAA ATAAGCGAAA AACGGAATTG AACCGATTAC CTTGGGAGCA 3600
 TGAATCCGAC GAACTTTCCT TATGCTCTAT TCGCTAAAT ACAATTAAAC TTGAAAAGAA 3660
 10 TTGAACTTTT ATTTTATAAT TCGTACTTAT ATATTTTATC CATTAAATTA CAAGTTCATT 3720
 ATATTATAAT ATATAAATTA TAAGTAATTA ACTTAGAGGT AAAGTTTCTG CTTTACATAC 3780
 15 AGAAGATCAT TGGTTCGATT CCAATATTAC TTAAATAAAT CTATAATTTA ATGGATAAAA 3840
 TAAAAACCTT CTAAGTTTTA TATGTAAGTT CAAATCTTAC TAGATTAAAT AATAATGAAT 3900
 ATGGCGAAAA GGTAACGCG CTAAATTTAG AATTTAGTTT TTATAATAAT AAGAGTTCGA 3960
 20 ATCTCTTTAT TCATATTTAT AATATACTTC TTAAACTAGG ATTGAACTAG TATCTTTCGG 4020
 TTAACAGCCG AATGCTTTAA CCACTAAGCT ATTAAGAATA TTAATATTAT ATTATATAAT 4080
 25 ATATAATAGG GAATATAGTT TAATGGTAAA ATCTTATTCT TGCATAATAA AGATAGTAGT 4140
 TCAATTCTAC TTATTTCCAT ATTATAAAAT CTATAAATGT TATAATTTTT AAATAATATA 4200
 TATATAATTA TATTGCGAGT TTGATCCTAG CTCAGAATGA ACGCTAGAAA TATACATTAC 4260
 30 ACATGCAAAT TTATGGATTA TATCATAGTG AATAGGTGAG GATATATAAA TTTTAAATTT 4320
 TAAATAGATT ATAATATATA ATAATCTATA AGCGCATTTA TTTATATAAT TGTACTATAT 4380
 35 TAAAAATTAT TATTGTTTAA AATAAAATTT ATATTTGATT AACTAGTTGG TAAAATAAAA 4440
 GCCTACCAAG GTTATGATCA AAAATTGGTT TTAAAGAATG TACAATCACA TTAGGGATTG 4500
 AAATAAGCC CTAAATTTTT TTAAATCAG CAGTGAGGAA TATTTTACAA TGAGCGTAAG 4560
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- 57 -

CTTGATAAAG TAATATTTCT TAAAGGATGA CAGTATATTT TTATATTGTA AACTTTATAT 4620

TTTATTTTTA AATATTGATA AAAATAAAAC ATTAGTATTT GCTAATTTCT GTGCCAGCAG 4680

5 CAGCGGTAAT ACAGAAAATA CCAGCGTTAT TCACTTTATT TGGCGTAAAG CGTTTAAAG 4740

TTTTATATTA ATTTTATTTT AAAATATTTA ATTTAAATTT GAATAAAAAA TAAATAATAA 4800

TATAATAAGA GTATTATAAA AGTATTAAGA ATTTTTTGAG AAGTAGTGAA ATGCAATGAT 4860

10 ACAAAAAAGA ATACCAAAGG CGAAGGCATA ATACTATATA ATAACTGACA CTTATAAACG 4920

AAAGCTAAGG TAGCAAATAG GATTAGATAC CCTAGTAGTC TTAGCTGTAA ACTATGAATA 4980

15 TTTTATATTT ATATATTAAT ATAAATATAA TAACTAACGT AATAAATATT CCGCCTGAGT 5040

AGTATATTCG CAAGAACGAA ATTCAAAGGA ATTGACGGGA GCTTATACAA GTGGTGGAAC 5100

ATGTGGCTTA ATTCGATGCA ACACGATAAA CCTTACCAA ATTTAACAAT ATTTTATTA 5160

20 TTAAGGAATT AATAGTTTAA TAAAATATAT AGGTAGTGCA TGGCTGTCGT CAGTTCGTGC 5220

TGTGAAGTAT TAATTTAAGT ATTATAACGA ACGTAACCCT TTTATAAAAA AAATTTTSTA 5280

25 TAATATATTT ATTAAATATA TAAAAAGAC TACGTCAAGT CATTATGCTC CTTATATTTT 5340

GGGCTGCTCA CGTGTTACAT AAAATATAAC AATATTTTAT TATATGAAAA TATAATATAT 5400

TAAATATATT TATAGTTCTG ATTATAAATT GAACTCATT TATATGAAGA TGAATCACT 5460

30 AGTAATCGCT AATAAGAAGT ATAGCGGTGA ATAAGTTCTT AAGCTTTGTA CACACCGCCC 5520

GTCACATCTG GAAAATATTA TATTATATAA AAATTATTGT AAAATAATAA TATATAATTA 5580

35 TATAATTTAG ATGAAGTCGT AACAGGTAG CCGTACTGGA AGGTGCGGCT GGATAATAAC 5640

ATAAAATTTT GGTGAATTA TTTATTTAAA AATAATATTT ATATATAAAA GTAATTATAA 5700

TTATATAATT TTTATAGACA AAAATAGCAT TAATACACAT TAATGTAAAT TTAGTTAAAT 5760

40

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ATTATTTTAT ATATATAAAG GTTTTtagTT TAATGGTAAA ACATACTCTT GATAAGGGTA 5820

AGACTTTAGT TCAATTCTAA AATAACCTA 5849

5 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 1711 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

15 (xi). SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCAGAAAA TAGGATTGTA ACCTATATTC TTCTATTCCC AAAATAGATA TGTTACCATT 60
ACACTATATT CTGAATATTT AAAATTTTAT ACTTTTAAGG AAAATCGAAT TCCTATTTTC 120
20 TTCTTGAAAA AAAGATGTCT TACCTTTAAA CGATAAAAGT AAAAAGTTAA ATTACCTGAG 180
ACTTGAAGTC AGAACCATTG GATTAAAAGT CGAGTACTCT ACCAATTAAG CTAGTAATTC 240
25 TTAATATAAC GAATCTGACG AGAATTGAAC TCGTATTCTT TGTTATGACA AAATAATATT 300
TTAACCTAAT TAACTACAA ATTCAAATAA ATATATATAG GGAAAAGGGA TTCGAACCCCT 360
GGTATATATA ATATCTACAT AAATGTAGCA ATTTATAGCT ATAACCACTC AGCCATTTCT 420
30 GTATATAATA ATAAGTTAAA TCAGATTGAA CTGATGTAGA TATAAAACCC AATGGATTTA 480
CAGTCCATCC CTTTAAACCC CTCAGGCATT AACTTTATTA TACATTTAAG TAGATTCGAA 540
35 CTAATGATGT TCAATATTTG AAAATGAATT ATGAGTCCAT TGCTTTTCGAC CTCTTAGCTA 600
TAAATGTTTA CTTTATTAGA GATAAAGGGA CTCGAACCCCT TACAACAATT ATTGTTAATG 660
GATTTTCTAA TTGAAATTTA GACTTTTTAT AAACATGTAT ATAAATAATA AAGTCGTTTG 720
40

- 59 -

AATATATAAC TAATATATTA CAGAATAAAA ATTATTTTTT CTTTATATAT ATTTAAATTA 780
 TTAATTTATT TATAAAATTA ACTCATAAAC AACGAATATA AATTATATTT ATATTATTTA 840
 5 AAGTCCATTG TGTATACCAA ATTCACCAT ATCTCTATTA TATACTATAT AAATGATATT 900
 CAGATTTGAA CTGAAATAAA ATAATTTGCA ATTATCCACT TTACCTAATT AAGTTATATC 960
 ATTATTATAT ATTATAAGAT AAATAAAGAG ATTGAACTC ATATAAAGA AACCACAATT 1020
 10 CCTTATCTTA ACCTTTAGGA TTATATTTAT CATTATTAA ACTTATTATA TATTATAAAT 1080
 ATTATTATAA ATATATAAAA TATTATTTAA ATATAAATCA TTTAATATTT TTATTTTAAA 1140
 15 ATTATATATA CATATAATAA AATTATCATT AAAACTAGAA GATTTAATAA AATTATATTT 1200
 ATATAAATTT GATATATAAA TATATATATT ATATCTATAA ATTAAATTTG GTGAAATTAT 1260
 ATATTTAATT TTTTATTAA AAAAAATTAT ATCCTTACCC TTTAATTTAA TATTATAATA 1320
 20 ATTACCATAA ACCTTATTTA AATATACATA TTTATACCTT ATATAATATC TCAGAGTGGT 1380
 GTATAGTTTT AAAAACCCCA TATTAACATA AAAGACATCT AATCTAGGTT CTAATAGATT 1440
 25 TAATAATTTG AGATATAAAT GATTCTCATG GTGACTCTGT ATTTTTTTCA AATAATGTAA 1500
 ATATGGTTTA AATGTTATAC CATAATTATA ACAGATATAT CTTACAAATT TTAATTTTAA 1560
 ATCGAAATAA GATTGATAGA CATATTTATT AATTTTAAAT TTATAATTAT ATTTACTAGA 1620
 30 TAAATATAAT AAAAAAGGAA GATTTAATTT TTTAACATT TTTATTTTAG GAGTTAAAAA 1680
 TTTTATCATA ATAATTTTAT ATTATAAAAT A 1711

35

- 60 -

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 516 base pairs

5 (B) TYPE: nucleic acid

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

10 (A) ORGANISM: Plasmodium berghei

(vii) IMMEDIATE SOURCE:

(B) CLONE: CLONE PRB

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAATAGACA TGGACATAAA GGTGTTATTT CTTATATTAA TGATATTAAT GATATGCCTT 60

20 ATTTAAATAA CAAAATACAA CCTGATTTAT TTGTAAGTGC TATTGGTATA CCTTCTAGAA 120

TAAATATAGG TCAAATATTA GAGGGTATAT ATGGATTAAA TAGTTTATAT TTAAATAATA 180

GATATATAAT ATCTAATAAT TTAAATACTA ATTATTATAA TAATTATATT AATAATTTTA 240

25 ATTATTATAA ATATAATTAT AATAATAATT TTGAATTCAA TAAAATATCA TATAATTATA 300

ATAAATATTT TTAAAAAAT CCGTTTACGG GCCATTTAAT ACAGAATAGT ATTTGTTTAA 360

30 ATAATATTTA TTATTATAAA TTAGTACATA TGGTAAAAGA TAAATTAAGA TATAGATTCA 420

TAGGATTATA TTCTGAATTA ACTCAACAAC CTGTAAAAGG AAATACAAAA CAAGGAGGTC 480

AAAGATTTGG TGAAATGGAA GTATGGGCGC TAGAAG 516

35

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 161 base pairs

5 (B) TYPE: nucleic acid

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

10 (A) ORGANISM: Plasmodium berghei

(vii) IMMEDIATE SOURCE:

(B) CLONE: CLONE PWQ

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTCAAAAAT CAGATTTGAC TGATAACACA TGGAAGTTCA ATCCATTGCT CTACCATTGA 60

20 GCTATAATGA CTTAATAATA TTATTATTAT AATAGAATAT AACCAAAAGG TTAAGGTAAT 120

GAACTTTGAT TTCATTAATA TAGGTTTCGAA TCCTTTAGGA C 161

25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

30 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GACCTGCATG AAAGATG

17

5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

20

GTATCGCTTT AATAGGCG

18

(2) INFORMATION FOR SEQ ID NO:7:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCCACTACTA TGAAAATC

18

40

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

15 GCGTTCATTC TGAGCTAG

18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

30

GCGGTAATAC AGAAAATGCA AGCG

24

35 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCACGAACT GACGACAGCC ATGCAC

26

10 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCAGGAATA CGTCTAGG

18

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCTAGTATTA TGTCTTCT

18

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTAGTATTA TGTCTTCA

18

15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGAATGTTAT TGCTAACAC

19

30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTAATCAATC TATGATAC

18

5

DATED this 26TH day of SEPTEMBER, 1997

The National University of Singapore

by DAVIES COLLISON CAVE

Patent Attorneys for the Applicants

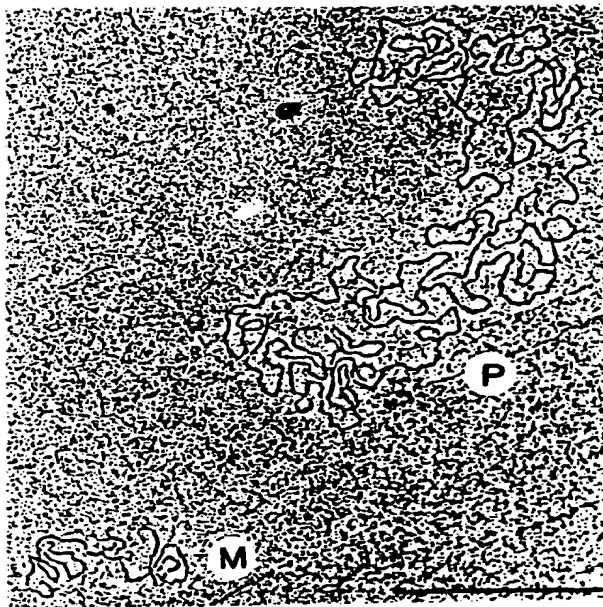


FIGURE 1

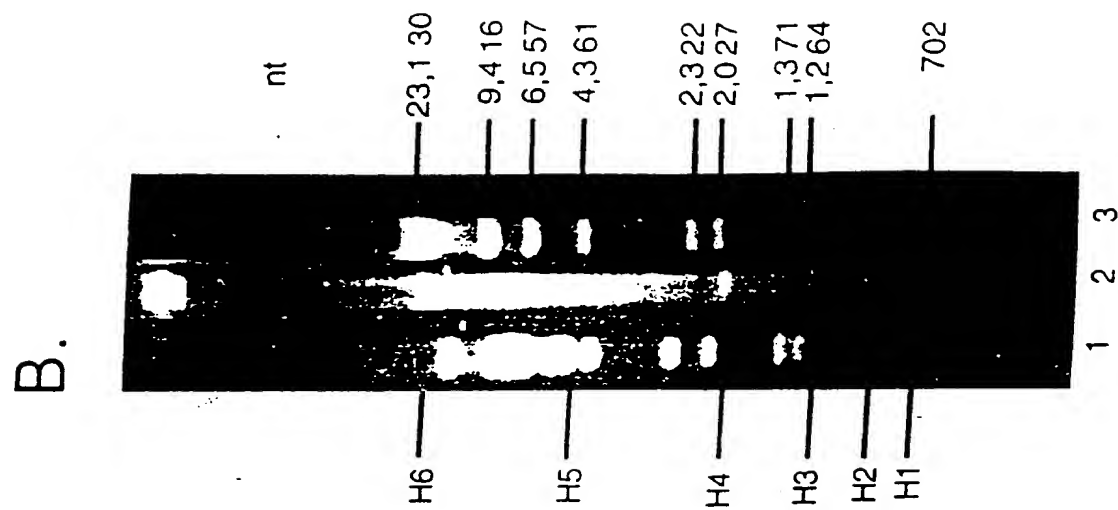
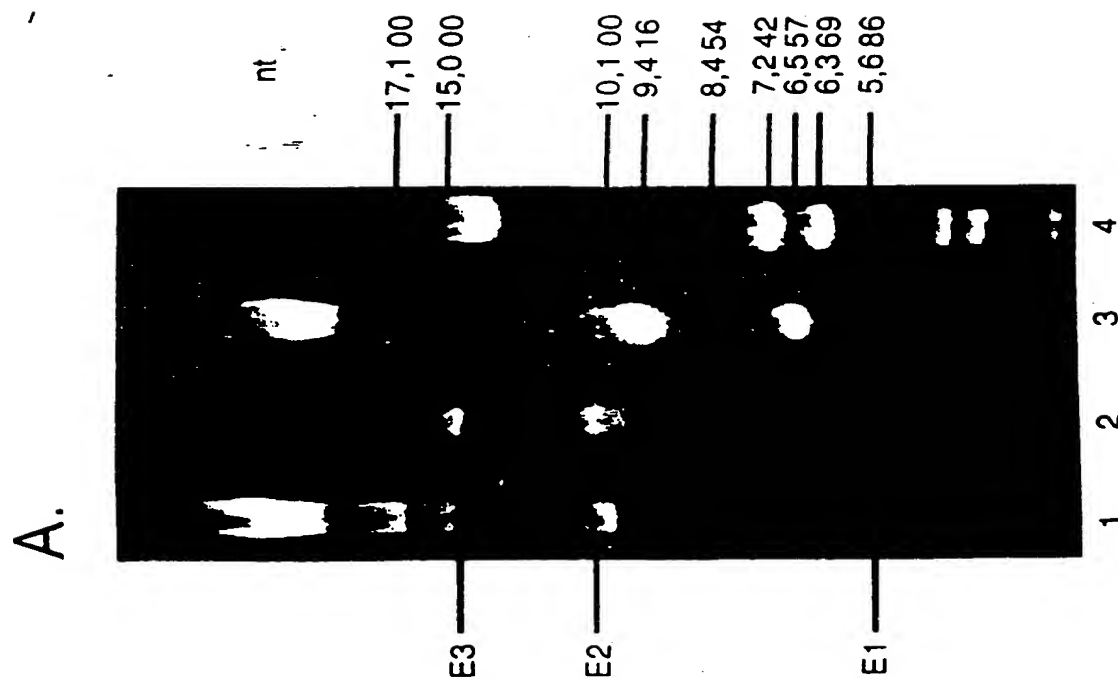


FIGURE 2

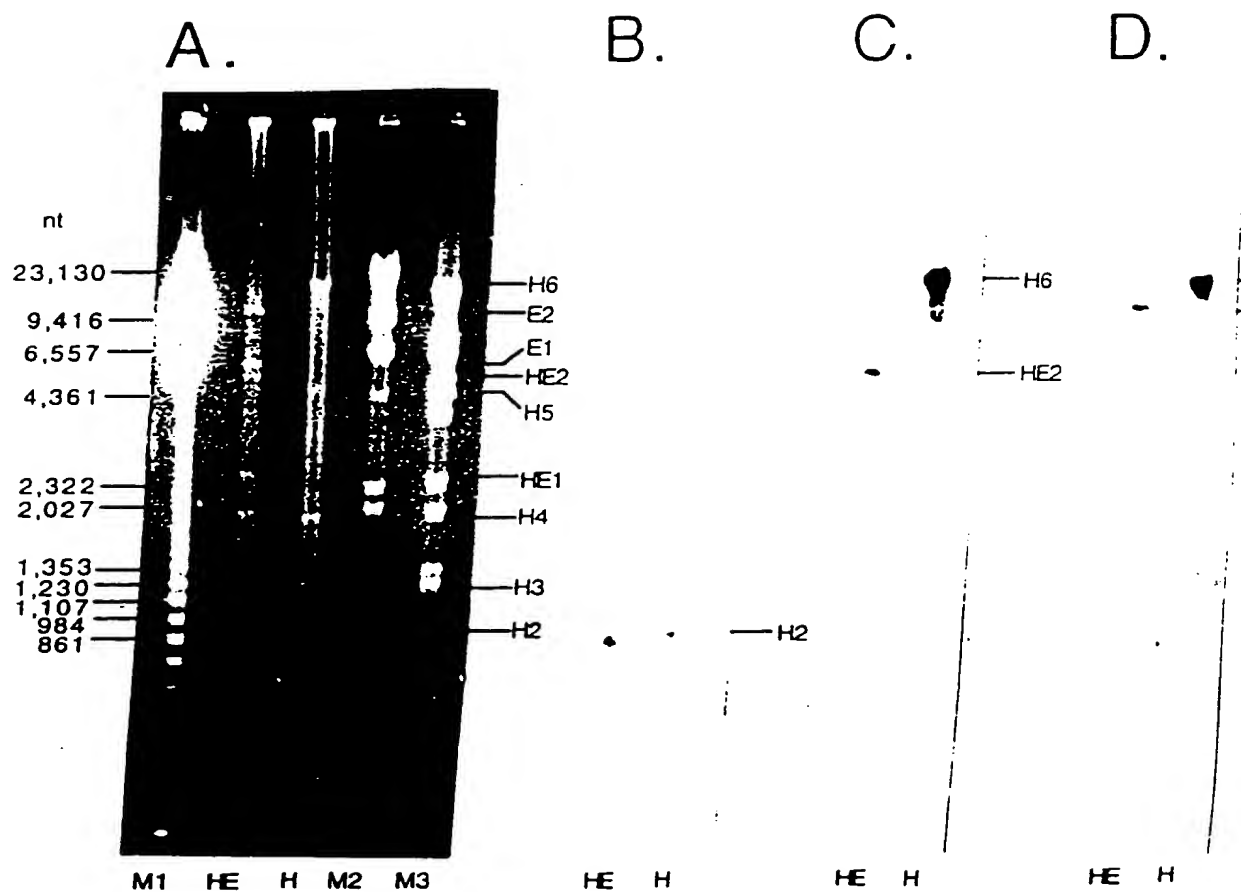


FIGURE 3

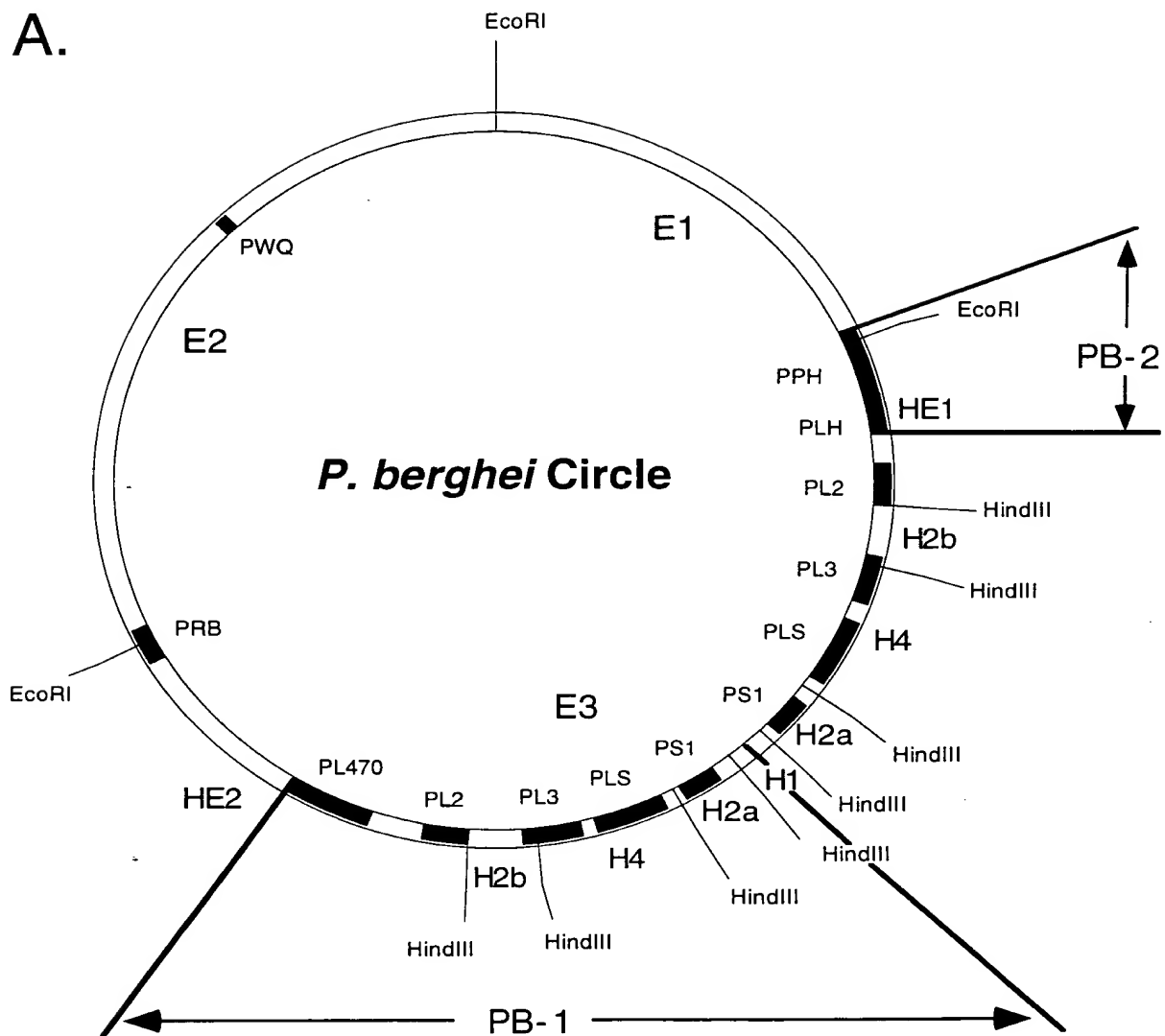


FIGURE 4A

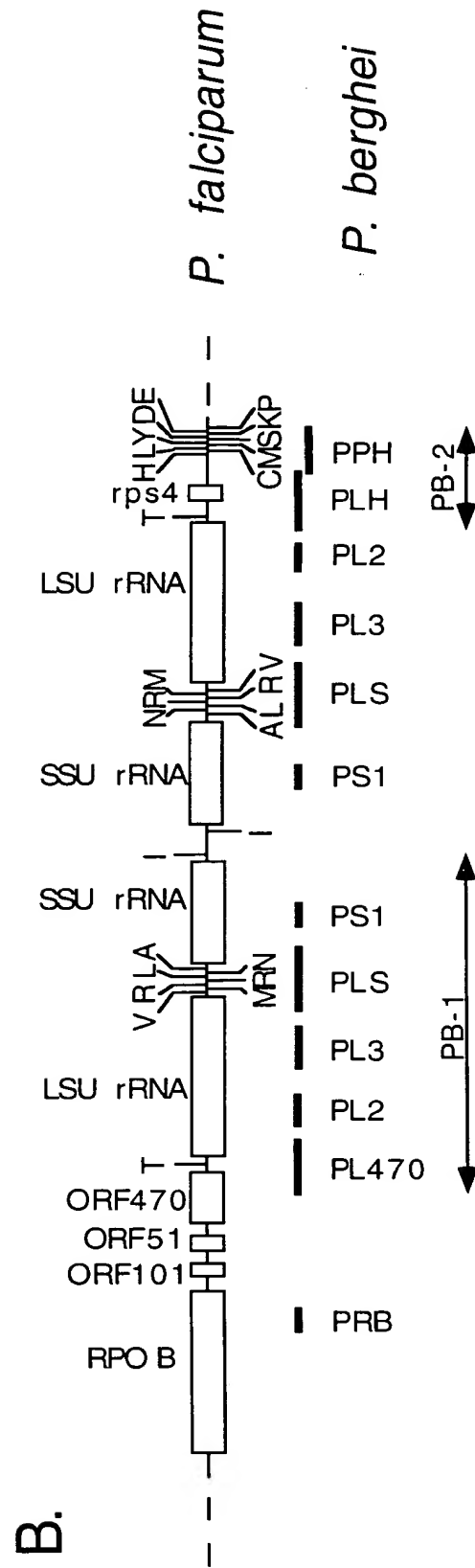


FIGURE 4B

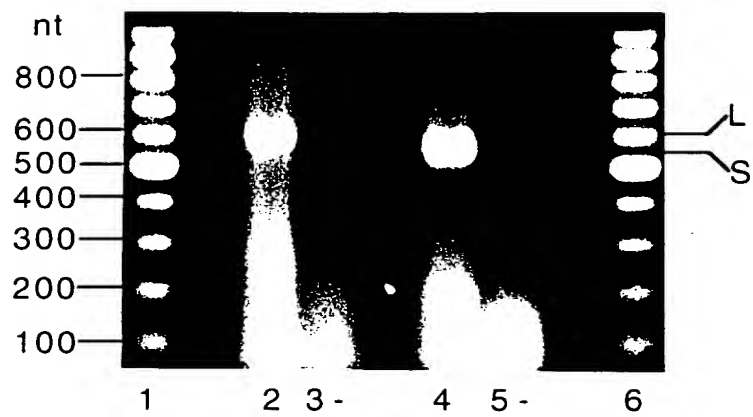


FIGURE 5

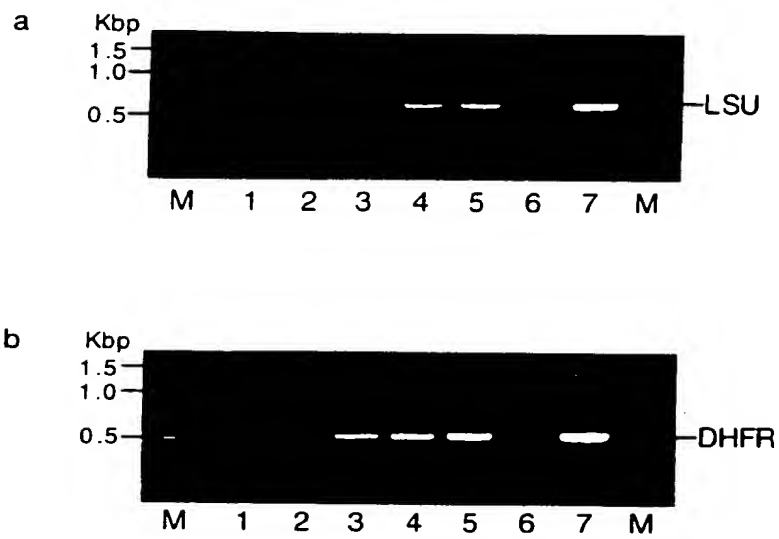


FIGURE 6



FIGURE 7

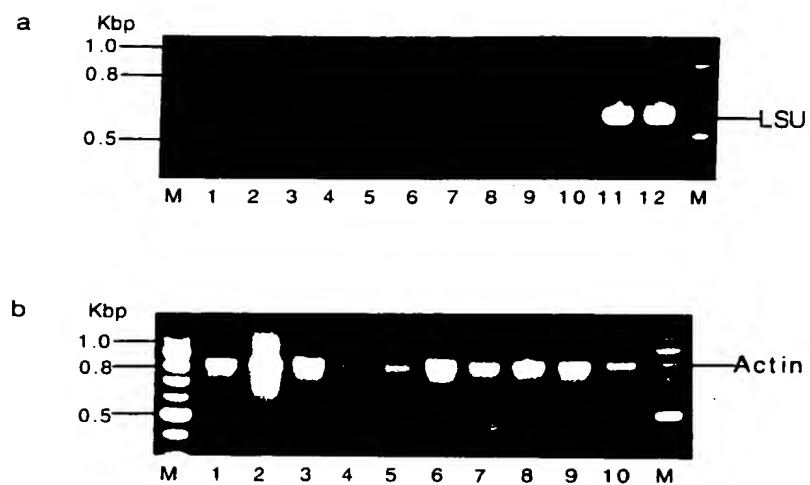


FIGURE 8

Friday, September 12, 1997 1:59 PM

1110	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf (C10)
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf10/P
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf11/P
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf19/I
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf20/L
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pf18/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv12/P
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv13/P
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv15/I
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv16/L
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv17/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pv86/C
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pm1/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pm38/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Po35/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Po36/S
1	GACCTGCATGAAAGATGTAACGACTTAAATGCTGCTTAAAAAATCTTAATGAAATAAAAATATATCTGTGAAGATACAGATTCTTATATTAGGACAC	Pb (ANKA)
1209	AAAGACCCCTATGAAGCTTTACTATTAAATAAATGAAATAATATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf (C10)
101	AAAGACCCCTATGAAGCTTTACTATTAAATAAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf10/P
100	AAAGACCCCTATGAAGCTTTTCTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf11/P
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf19/I
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf20/L
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pf18/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv12/P
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv13/P
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv15/I
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv16/L
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv17/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pv86/C
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pm1/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pm38/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Po35/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Po36/S
100	AAAGACCCCTATGAAGCTTTTACTATTAAATAAATAATGAAATAATATATATTTAACATAGTATAAATGGGAAACAATAATATTATTTCTTGGAAATAATTT	Pb (ANKA)

FIGURE 9A


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309 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf (C10)
01 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-GAATTTTAAACAACTATTTATGAGATAGTTTGACTGGG-C Pf10/P
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf11/P
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf19/I
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf20/L
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf18/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf12/P
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf13/P
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf15/I
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf16/L
00 AGTTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf17/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pf86/C
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pm1/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pm38/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Po35/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Po36/S
00 AAATTGTAAATGAAATACCATTTTATTTATATATAAATCTCTATAGAAATTTTATAACA-AAATTTTAAACAA-TATTTATGAGATAGTTTGACTGGG-C Pb (ANKA)

1406 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf (C10)
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf10/P
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf11/P
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf19/I
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf20/L
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf18/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf12/P
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf13/P
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf15/I
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf16/L
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf17/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pf86/C
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pm1/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pm38/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Po35/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Po36/S
00 GGTCCTCCTCTATATAAAACGGAGGAGTACAATGTTTATATTTATTTATATAAAGATATAATATAATTAACCTGTAAATTTACAAATTTAAACACAGAGATA Pb (ANKA)

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FIGURE 9B

4506	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAG;I:C	Pf (C10)
400	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pf10/P
397	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pf11/P
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pf19/I
397	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pf20/L
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pf18/S
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv12/P
397	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv13/P
397	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv15/I
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv16/L
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv17/S
395	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pv86/C
397	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pm1/S
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Pm38/S
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Po35/S
396	AATGTCGGTCTTAATGATCCGATAAATTAATTTAGTAATAAATAATATCGCTTAACGGATAAAAGTTACTCTAGGGATAACAGGCTAATCTTTTCCGAGAGTC	Po36/S
4606	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf (C10)
500	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf10/P
497	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf11/P
496	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf19/I
497	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf20/L
496	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pf18/S
496	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv12/P
497	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv13/P
497	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv15/I
496	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv16/L
496	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv17/S
495	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pv86/C
197	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pm1/S
196	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Pm38/S
196	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Po35/S
196	CATATTGACGAAAAGGTTTGGCACCTCGATGTCGGCTTATCGCATCTTAAGCAGTAGTAGTATGTTTTAAGGTAAGTCTGTT-CGCCATATTAAGCGGATAC	Po36/S

Decoration 'Decoration #1': Box residues that differ from Pf(C10).

FIGURE 9C

1	GACTGATGGATCAAAATATTTCTCATTTATATCCGAGCCTCA-----TGTTA-----TTTTTATTGTTTTAAATAGATATTCACCT	PfcoxI
1	GACTGATGGATCGAAATCTTTATTCATATCCAGCCCTCACTTAATGTTAATTATATATATATATTTTTTTTCTGTTTCCAAATAGATATTCACCT	Pv15coxI
1	GACTGATGGATCGAAATCTTTATTCATATCCAGCCCTCACTTAATGTTAATTATATATATATATTTTTTTTCTGTTTCCAAATAGATATTCACCT	Pv16coxI
1	GACTGATGGATCGAAATCTTTATTCATATCCAGCCCTCACTTAATGTTAATTATATATATATATTTTTTTTCTGTTTCCAAATAGATATTCACCT	Pv32coxI
1	GACTGATGGATCGAAATCTTTATTCATATCCAGCCCTCACTTAATGTTAATTATATATATATATTTTTTTTCTGTTTCCAAATAGATATTCACCT	Pv37coxI
16	TATTACAAATGTAACCATATAAACTTTTAGGATTATACATATTTATGTTTTCATTTTTTATTTGGTAGTTATGGATTTTTTTTATTATCAGTAATACTAC	PfcoxI
16	TATTACAAATGTAACCATATAAACTTTTAGGATTATACATATTTATGTTTTCATTTTTTATTTGGTAGTTATGGTTTTTTTATTATCTGTATATTTTAC	Pv15coxI
14	TATTACAAATGTAACCATATAAACTTTTAGGATTATACATATTTATGTTTTCATTTTTTATTTGGTAGTTATGGTTTTTTTATTATCTGTATATTTTAC	Pv16coxI
15	TATTACAAATGTAACCATATAAACTTTTAGGATTATACATATTTATGTTTTCATTTTTTATTTGGTAGTTATGGTTTTTTTATTATCTGTATATTTTAC	Pv32coxI
14	TATTACAAATGTAACCATATAAACTTTTAGGATTATACATATTTATGTTTTCATTTTTTATTTGGTAGTTATGGTTTTTTTATTATCTGTATATTTTAC	Pv37coxI
171	GTACTGAATTAATATTCCTTTCATCTTTAAAGATAAATGTCACAAGAAAAATGTAATCTATATAATAATATGATATTTTACAATTCACGGAAATAATTTATGATT	PfcoxI
191	GTACAGAAATTAATATTCCTTCTTTAAAGATAAATGTCACAAGAAAAATGTAATCTATATAATAATATGATATTTTACAATTCACGGAAATAATTTATGATT	Pv15coxI
189	GTACAGAAATTAATATTCCTTCTTTAAAGATAAATGTCACAAGAAAAATGTAATCTATATAATAATATGATATTTTACAATTCACGGAAATAATTTATGATT	Pv16coxI
190	GTACAGAAATTAATATTCCTTCTTTAAAGATAAATGTCACAAGAAAAATGTAATCTATATAATAATATGATATTTTACAATTCACGGAAATAATTTATGATT	Pv32coxI
189	GTACAGAAATTAATATTCCTTCTTTAAAGATAAATGTCACAAGAAAAATGTAATCTATATAATAATATGATATTTTACAATTCACGGAAATAATTTATGATT	Pv37coxI
266	TTTTTCAATATAAATGCCAGGATTAATTCGGAGGATTTGGTAATTAATTAATCTTCTTACCTATTTTATGTTGGATCTCCAGAAATTAGCATATTCCTAGAAATTAA	PfcoxI
286	TTCTTTAAATATAATGCCAGGATTAATTCGGAGGATTTGGTAATTAATTAATTAATCTTCTTACCTATTTTATGTTGGTCTCTCCAGAAATTCGCATATCCAGAAATTAA	Pv15coxI
284	TTCTTTAAATATAATGCCAGGATTAATTCGGAGGATTTGGTAATTAATTAATTAATTAATCTTCTTACCTATTTTATGTTGGTCTCTCCAGAAATTCGCATATCCAGAAATTAA	Pv16coxI
285	TTCTTTAAATATAATGCCAGGATTAATTCGGAGGATTTGGTAATTAATTAATTAATTAATTAATCTTCTTACCTATTTTATGTTGGTCTCTCCAGAAATTCGCATATCCAGAAATTAA	Pv32coxI
284	TTCTTTAAATATAATGCCAGGATTAATTCGGAGGATTTGGTAATTAATTAATTAATTAATTAATTAATCTTCTTACCTATTTTATGTTGGTCTCTCCAGAAATTCGCATATCCAGAAATTAA	Pv37coxI
361	TAGTATATCTTTACTGTTACAACCAATAGCTTTTGTGTTTAGTTATATATCTACTGCAGCAGAAATTTGGTGGTGGAACTGGATGGACTTTTATATC	PfcoxI
381	TAGTATATCTTTACTGTTACAACCAATAGCTTTTGTGTTTAGTTATATATCTACTGCAGCAGAAATTTGGTGGTGGAACTGGATGGACTTTTATATC	Pv15coxI
379	TAGTATATCTTTACTGTTACAACCAATAGCTTTTGTGTTTAGTTATATATCTACTGCAGCAGAAATTTGGTGGTGGAACTGGATGGACTTTTATATC	Pv16coxI
380	TAGTATATCTTTACTGTTACAACCAATAGCTTTTGTGTTTAGTTATATATCTACTGCAGCAGAAATTTGGTGGTGGAACTGGATGGACTTTTATATC	Pv32coxI
379	TAGTATATCTTTACTGTTACAACCAATAGCTTTTGTGTTTAGTTATATATCTACTGCAGCAGAAATTTGGTGGTGGAACTGGATGGACTTTTATATC	Pv37coxI
156	CACCAATTAAGTACATCTTTAATGTCAATATATCTCTGTAGTGTAAATATTTTGGTTTATAGTATCTCGAGTCGCTAGTATATGTCT	PfcoxI
176	CACCAATTAAGTACATCACTTATGTCTTTATCTCCTGTGAGTAGATGTATCATTTGTTGGTCTTTAGTATCTGGTATTCGCTAGTATATGTCT	Pv15coxI
174	CACCAATTAAGTACATCACTTATGTCTTTATCTCCTGTGAGTAGATGTATCATTTGTTGGTCTTTAGTATCTGGTATTCGCTAGTATATGTCT	Pv16coxI
175	CACCAATTAAGTACATCACTTATGTCTTTATCTCCTGTGAGTAGATGTATCATTTGTTGGTCTTTAGTATCTGGTATTCGCTAGTATATGTCT	Pv32coxI
174	CACCAATTAAGTACATCACTTATGTCTTTATCTCCTGTGAGTAGATGTATCATTTGTTGGTCTTTAGTATCTGGTATTCGCTAGTATATGTCT	Pv37coxI
551	TCATTTAAATTTTATTACTACAGTAATGCAATTAAGAGCAAAAGGATTAACACTTGGTATATTTAAGTGTCTTCTACATGGTCAATGATCATACATC	PfcoxI
571	TCCTTTAAATTTTATTACTACTGTAATGTCATCTAAGATCTAAAGGTTTAAACACTTGGTATATTTAAGTGTCTTCTACATGGTCAATGATCATACATC	Pv15coxI
569	TCCTTTAAATTTTATTACTACTGTAATGTCATCTAAGATCTAAAGGTTTAAACACTTGGTATATTTAAGTGTCTTCTACATGGTCAATGATCATACATC	Pv16coxI
570	TCCTTTAAATTTTATTACTACTGTAATGTCATCTAAGATCTAAAGGTTTAAACACTTGGTATATTTAAGTGTCTTCTACATGGTCAATGATCATACATC	Pv32coxI
569	TCCTTTAAATTTTATTACTACTGTAATGTCATCTAAGATCTAAAGGTTTAAACACTTGGTATATTTAAGTGTCTTCTACATGGTCAATGATCATACATC	Pv37coxI

FIGURE 10A

646	AGGAATGTTATTGCTAAACACACTACCGGTTTAACTGGAGGAGTATTAATGTATTATATCAGACTTACATTTTAAATACATTTTATTTTGTGACCCACACAT	P15coxI
666	TGTAATGCTATTATTAACATTAACCTGTTTAAACAGGTGGTGTGTTTAAATGTTATATATCAGATTTACATTTTAAATACATTTTATTTTGTGACAT	Pv15coxI
664	TGTAATGCTATTATTAACATTAACCTGTTTAAACAGGTGGTGTGTTTAAATGTTATATATCAGATTTACATTTTAAATACATTTTATTTTGTGACAT	Pv16coxI
665	TGTAATGCTATTATTAACATTAACCTGTTTAAACAGGTGGTGTGTTTAAATGTTATATATCAGATTTACATTTTAAATACATTTTATTTTGTGACAT	Pv32coxI
664	TGTAATGCTATTATTAACATTAACCTGTTTAAACAGGTGGTGTGTTTAAATGTTATATATCAGATTTACATTTTAAATACATTTTATTTTGTGACAT	Pv37coxI
741	TTGCAGGAGATCCAAATATTATATCAACATTTATCTGGTATTATTTGGACATCCTGAAGTATACATTTTAAATATACCTGCTTTTGGAGTAAATTAGT	PfcoxI
761	TTGCTGGAGATCCATTTTATATCAACATCTATTTGGTATTATTTGGATCCTGAAGTGTATTTTAAATATACCTGCTTTTGGTGTGTTTATTTAGT	Pv15coxI
759	TTGCTGGAGATCCATTTTATATCAACATCTATTTGGTATTATTTGGATCCTGAAGTGTATTTTAAATATACCTGCTTTTGGTGTGTTTATTTAGT	Pv16coxI
760	TTGCTGGAGATCCATTTTATATCAACATCTATTTGGTATTATTTGGATCCTGAAGTGTATTTTAAATATACCTGCTTTTGGTGTGTTTATTTAGT	Pv32coxI
759	TTGCTGGAGATCCATTTTATATCAACATCTATTTGGTATTATTTGGATCCTGAAGTGTATTTTAAATATACCTGCTTTTGGTGTGTTTATTTAGT	Pv37coxI
836	CATGTAATTTCTACTAATATTATGCAGAAATCTATTTGGTAAATCAATCTATGATCTTGCTATGGGATGTATAGCTGTTTATAGGAAGCTTAGTATG	PfcoxI
856	CATGTAATTTCTACTAATATTATGCAGAAATCTATTTGGTAAATCAATCTATGATCTTGCTATGGGATGTATAGCTGTTTATAGGAAGCTTAGTATG	Pv15coxI
854	CATGTAATTTCTACTAATATTATGCAGAAATCTATTTGGTAAATCAATCTATGATCTTGCTATGGGATGTATAGCTGTTTATAGGAAGCTTAGTATG	Pv16coxI
855	CATGTAATTTCTACTAATATTATGCAGAAATCTATTTGGTAAATCAATCTATGATCTTGCTATGGGATGTATAGCTGTTTATAGGAAGCTTAGTATG	Pv32coxI
854	CATGTAATTTCTACTAATATTATGCAGAAATCTATTTGGTAAATCAATCTATGATCTTGCTATGGGATGTATAGCTGTTTATAGGAAGCTTAGTATG	Pv37coxI
931	GGTACATCATATGTACACTACTTGTTTGAAGTTGATCTAGAGCTTATTTTACTTCGACTACCATTTTAAATATCAATCACTACCTACCGGTACAAAAA	PfcoxI
951	GGCTCATCATATGTATACTACTAGTTTGAAGTTGATCTAGAGCTTATTTTACTTCGACTACCATTTTAAATATCAATCACTACCTACCGGTACAAAAA	Pv15coxI
949	GGCTCATCATATGTATACTACTAGTTTGAAGTTGATCTAGAGCTTATTTTACTTCGACTACCATTTTAAATATCAATCACTACCTACCGGTACAAAAA	Pv16coxI
950	GGCTCATCATATGTATACTACTAGTTTGAAGTTGATCTAGAGCTTATTTTACTTCGACTACCATTTTAAATATCAATCACTACCTACCGGTACAAAAA	Pv32coxI
949	GGCTCATCATATGTATACTACTAGTTTGAAGTTGATCTAGAGCTTATTTTACTTCGACTACCATTTTAAATATCAATCACTACCTACCGGTACAAAAA	Pv37coxI
1026	TATTTAACTGGATATGTACATATATAGTAGTAGTAATTTTGGTATGATACACAGCTCTTCATTTATTTGTCAATTTATTTATATGTACATTTTACATTT	PfcoxI
1046	TATTTAACTGGATATGTACATATATAGTAGTAGTAATTTTGGTATGATCTCAATGTTCTCTTTTATTTATTTATTTATATGTACATTTTACATTT	Pv15coxI
1044	TATTTAACTGGATATGTACATATATAGTAGTAGTAATTTTGGTATGATCTCAATGTTCTCTTTTATTTATTTATTTATTTATGTACATTTTACATTT	Pv16coxI
1045	TATTTAACTGGATATGTACATATATAGTAGTAGTAATTTTGGTATGATCTCAATGTTCTCTTTTATTTATTTATTTATTTATGTACATTTTACATTT	Pv32coxI
1044	TATTTAACTGGATATGTACATATATAGTAGTAGTAATTTTGGTATGATCTCAATGTTCTCTTTTATTTATTTATTTATTTATGTACATTTTACATTT	Pv37coxI
1121	GGAGGTACTACGGAGTTATATTAGGTAATGCTGCCATTGATGTAGCATTTACATGACACATATTTATGTTATTTGCTCATTTCCATTTTGTGACTATC	PfcoxI
1141	GGTGGTACTACGGAGTTATATTAGGTAATGCTGCCATTGATGTAGCATTTGATGATTTGCTCATTTCCATTTCCATTTTGTGATTTATC	Pv15coxI
1139	GGTGGTACTACGGAGTTATATTAGGTAATGCTGCCATTGATGTAGCATTTGATGATTTGCTCATTTCCATTTCCATTTTGTGATTTATC	Pv16coxI
1140	GGTGGTACTACGGAGTTATATTAGGTAATGCTGCCATTGATGTAGCATTTGATGATTTGCTCATTTCCATTTCCATTTTGTGATTTATC	Pv32coxI
1139	GGTGGTACTACGGAGTTATATTAGGTAATGCTGCCATTGATGTAGCATTTGATGATTTGCTCATTTCCATTTCCATTTTGTGATTTATC	Pv37coxI
1216	AATTTGGTCAATATTGGATTATTACAACTGTAAGTGCAATTTCAAGATAAATTTCTTTTGGTAAACCTTACGTGAAATTTCTATTTGTAATACATAT	PfcoxI
1236	TATTTGGTCAATATTGGATTATTACAACTGTAAGTAGTTTCAAGATAAATTTTGGTAAACCTTACGTGAAATTTCTATTTGTAATACATAT	Pv15coxI
1234	TATTTGGTCAATATTGGATTATTACAACTGTAAGTAGTTTCAAGATAAATTTTGGTAAACCTTACGTGAAATTTCTATTTGTAATACATAT	Pv16coxI
1235	TATTTGGTCAATATTGGATTATTACAACTGTAAGTAGTTTCAAGATAAATTTTGGTAAACCTTACGTGAAATTTCTATTTGTAATACATAT	Pv32coxI
1234	TATTTGGTCAATATTGGATTATTACAACTGTAAGTAGTTTCAAGATAAATTTTGGTAAACCTTACGTGAAATTTCTATTTGTAATACATAT	Pv37coxI

FIGURE 10B

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1311 GGTC AATGTTATATTTTGTAGGTGTAATAATAACATTTTACCTATGCAATTTTTAGGATTTAAATGTAATGCCCTAGACGTAATTCCTGATTATCCA P1coxI
1331 GGTC AATGTTATATTTTATTTGGAGCTGTATTAACATTTTACCTATGCAATTTTGGGATTTAAATGTAATGCCCTAGACGTAATTCCTGATTATCCA P15coxI
1329 GGTC AATGTTATATTTTATTTGGAGCTGTATTAACATTTTACCTATGCAATTTTGGGATTTAAATGTAATGCCCTAGACGTAATTCCTGATTATCCA P16coxI
1330 GGTC AATGTTATATTTTATTTGGAGCTGTATTAACATTTTACCTATGCAATTTTGGGATTTAAATGTAATGCCCTAGACGTAATTCCTGATTATCCA P16coxI
1329 GGTC AATGTTATATTTTATTTGGAGCTGTATTAACATTTTACCTATGCAATTTTGGGATTTAAATGTAATGCCCTAGACGTAATTCCTGATTATCCA P16coxI
1406 GACGCTTTAAATGGATGGAATATATGATTTGTTCTATTTGGGTCAACAATGACTTTTATTTGGTTTACTAAATTTTAAATAATATAC-TATTTATTGT P1coxI
1426 GACGCTTTAAATGGATGGAATATATGATTTGTTCAATTTGGATCAACAATGACTTTTATTTGGTTTATTTATTTTAAATAATATATAATTTTGT P15coxI
1424 GACGCTTTAAATGGATGGAATATATGATTTGTTCAATTTGGATCAACAATGACTTTTATTTGGTTTATTTATTTTAAATAATATATAATTTTGT P16coxI
1425 GACGCTTTAAATGGATGGAATATATGATTTGTTCAATTTGGATCAACAATGACTTTTATTTGGTTTATTTATTTTAAATAATATATAATTTTGT P16coxI
1424 GACGCTTTAAATGGATGGAATATATGATTTGTTCAATTTGGATCAACAATGACTTTTATTTGGTTTATTTATTTTAAATAATATATAATTTTGT P16coxI
1500 TTTTATGAACCTTTTACTCTATTAATTTAGTTAAAGCACACTTAATAAATACCCCATGTCCATTG P1coxI
1521 TTTTATGAACCTTTTATTTCTATTAATTTAGCAAAAAGCACATTTATTTAAATTTACCCCATGTCCATTG P15coxI
1519 TTTTATGAACCTTTTATTTCTATTAATTTAGCAAAAAGCACATTTATTTAAATTTACCCCATGTCCATTG P16coxI
1520 TTTTATGAACCTTTTATTTCTATTAATTTAGCAAAAAGCACATTTATTTAAATTTACCCCATGTCCATTG P16coxI
1519 TTTTATGAACCTTTTATTTCTATTAATTTAGCAAAAAGCACATTTATTTAAATTTACCCCATGTCCATTG P16coxI
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Decoration 'Decoration #1': Box residues that differ from pfcoxI.

FIGURE 10C

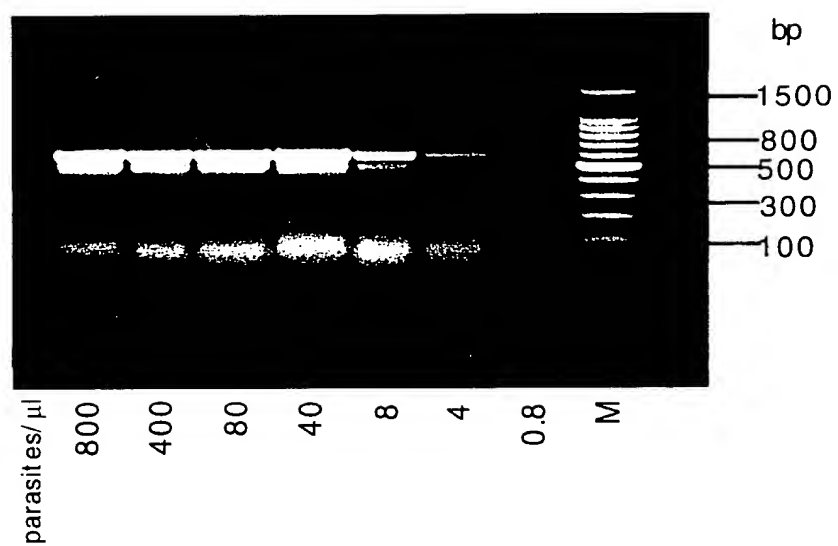


FIGURE 11